E & A

Mosquito News

JOURNAL OF
THE
AMERICAN MOSQUITO CONTROL ASSOCIATION

VOLUME 21 SEPTEMBER, 1961 NUMBER 3

COMMONWEALTH
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U.S.A. 146

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Annual Meeting, March 5, 6, 7, 1962, Galveston, Texas

MOSQUITO NEWS

Journal of the American Mosquito Control Association

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Published by

AMERICAN MOSQUITO CONTROL ASSOCIATION, INC.

Printed by

Boyd Printing Company, Inc., Albany, N. Y.

A quarterly journal published on the 20th of March, June, September and December. Devoted to the improvement and the sound extension of mosquito control and related work as a vital public health service.

SUBSCRIPTION RATES

$5.00 per Year (U. S. money), Single Copies, $1.75
Mailed without charge to members of the Association.

MEMBERSHIP DUES—$5.00

PUBLICATION OFFICE: American Mosquito Control Association, Inc., Room 976, State Education Bldg., Albany 1, N. Y. GENERAL OFFICE: P. O. Box 278, Selma, Calif. BUSINESS OFFICE: P. O. Box 346, Metuchen, N. J. *Second class postage* paid at Albany, N. Y.

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Communications relating to editorial matters and manuscripts submitted for publication in MOSQUITO NEWS should be addressed to Donald L. Collins, Editor, MOSQUITO NEWS, New York State Museum, Albany 1, New York.

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Communications relating to subscriptions to MOSQUITO NEWS, Membership in the American Mosquito Control Association, and other association matters should be addressed to T. G. Raley, Executive Secretary, American Mosquito Control Association, P. O. Box 278, Selma, Calif. Changes of address should reach the Secretary before the first of the month of publication.

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ARTICLES

HOST PREFERENCES OF *CULISETA MELANURA* AND ALLIED MOSQUITOES [1]

RICHARD O. HAYES [2]

The role of mosquitoes as vectors of eastern encephalitis (EE) is at present poorly known. In Massachusetts, EE virus has been isolated from only one species of naturally infected mosquito, *Culiseta melanura* (1). This arthropod is generally considered to be the principal enzootic vector of EE (2), but not in all areas (3). Other mosquitoes or even other arthropods are thought to transmit the virus to man and horse (2, 4). Among the mosquitoes occurring in Massachusetts that may be involved in EE transmission are five species which have been found naturally infected elsewhere: *Mansonia perturbans* (5), *Anopheles crucians* (6), *Culex salinarius* (7), *Culex restuans* (8), and *Aedes vexans* (9, 10).

The general hypothesis regarding transmission of EE is that wild birds are the principal reservoirs, though it appears from laboratory data (11) and field data (12) that small mammals could also be involved as reservoirs. Presumably any mosquitoes or other haematophagous arthropods that serve as primary enzootic vectors of EE would show a high preference for feeding on birds, and any endemic vectors that transmit EE to man and domestic animals would be species which feed readily on mammals as well as birds. The primary objective of the present study was to obtain information on the relative attractiveness of various species of amphibians, reptiles, birds and mammals for *C. melanura* and associated mosquitoes. Previous investigations (13) had shown that if potential hosts were exposed in traps and the bait animal were accessible to the trapped mosquitoes for feeding, the hosts could be compared with respect to both the number of mosquitoes attracted and the percent of mosquitoes which obtained blood.

MATERIALS AND METHODS. The observations were made between July 6 and September 12, 1959, in Raynham, Massachusetts, at a rural residence with a long history of EE virus activity. The exact site was less than 200 yards from Hockomock Swamp, the largest freshwater swamp in the state. The animals used as bait in the mosquito traps were obtained in Raynham or other nearby towns. They included seven species of birds, eight of mammals, six of reptiles, and four of amphibians (Table 1).

The cylindrical traps, operated in a horizontal position, were made from lard cans of 120-pound capacity (Fig. 1). They were similar to previous bait traps (13, 14) with two exceptions: the dimensions of the conical entrance baffles placed in the two ends, and the device for holding the test animal. The baffles, made of galvanized wire screening of 14 x 18 mesh, were 10½ inches in diameter at the base and 8½ inches high. The entrance holes, each ¾ inch in diameter and reinforced by a metal ring to insure permanence of

[1] This investigation is part of a joint study on eastern encephalitis by the Division of Communicable Disease, Massachusetts Department of Public Health, and the Communicable Disease Center, U. S. Public Health Service. The assistance of Mr. Frank M. Mack and Mr. Robert Frazer in the field and laboratory phases of this study, the statistical counsel provided by Dr. Richard G. Cornell and Mr. Elmer C. Hall, and the analytical review of the data by Dr. Richard P. Dow and Dr. A. D. Hess are gratefully acknowledged.

[2] Encephalitis Section, Technology Branch, Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Taunton, Massachusetts.

TABLE 1.—Attraction and engorgement of four common mosquito species in tests with twenty-five vertebrate species conducted at Raynham, Massachusetts during 1959 [1]

Vertebrate species	No. of trap nights	*Culiseta melanura*		*Culex pipiens*		*Culex salinarius*		*Aedes canadensis*	
		A.[2]	E.[3]	A.	E.	A.	E.	A.	E.
Birds									
Catbird	1	63	0	..	..	109	29	19	10
Chicken, 4–5 week	11	66	85	58	94	27	70	12	97
Grackle, common	5	116	64	99	78	3	90	11	98
Pheasant, immature	7	195	80	106	86	81	93	12	86
Pigeon, domestic	5	255	38	92	88	150	45	52	46
Sparrow, English	2	87	32	..	..	188	48	3	100
Starling, immature	3	299	21	..	..	87	48	25	13
All birds	34	153	55	86	85	76	57	11	58
Mammals									
Bat, little brown	4	25	0	18	18	10	0	2	50
Chipmunk, eastern	4	75	9	..	..	92	48	40	42
Mouse, white-footed	4	24	0	10	0	49	42	8	55
Rabbit, eastern cottontail	6	63	28	27	46	120	55	6	93
Rabbit, domestic	4	9	25	..	..	8	58	8	90
Rat, Norway	4	22	11	..	..	8	63	27	46
Squirrel, eastern gray	4	89	14	37	23	112	33	7	100
Vole, redback	5	64	1	24	19	47	7	1	80
All mammals	35	48	12	24	26	66	45	18	62
Reptiles									
Snake, eastern garter	4	2	0	0	0	7	0	2	11
Snake, eastern milk	4	1	100	3	61	0	0	0	0
Snake, northern water	8	10	35	15	56	19	54	1	50
Turtle, eastern box	4	9	0	20	28	24	46	1	0
Turtle, eastern painted	4	1	0	0	0	9	17	5	74
Turtle, eastern spotted	4	1	0	..	..	6	29	11	91
All reptiles	28	5	21	10	49	14	41	2	69
Amphibians									
Bullfrog	4	8	0	..	..	46	11	3	22
Frog, green	4	20	0	..	..	22	16	19	12
Salamander, redback	4	10	0	..	..	6	0	11	0
Toad, American	4	1	0	0	0	5	0	4	0
All amphibians	16	10	0	0	0	20	11	10	8

[1] Total number of trap nights when each mosquito was present was: *C. melanura,* 113; *C. pipiens,* 47; *C. salinarius,* 95; *A. canadensis,* 109.

[2] Average number attracted per trap night when at least one specimen of the species was taken in any trap. Dashes indicate mosquito species absent during all trials with the vertebrate.

[3] Percent of mosquitoes engorged (all trap nights combined). Dashes indicate mosquito species absent during all trials with the vertebrate.

the aperture size, were about 3½ inches apart when the baffles were in position. The pocket for holding the test animal was 10½ inches wide by 6½ inches high. It was made of nylon mesh (28 threads to the inch) and provided with a 2-inch muslin collar with four button holes. It could be inserted and removed through a hole, 6 inches in diameter, which was cut in the top of the trap. The pocket, placed in a transverse position in the trap, was supported by flaring the collar over the outer surface, holding it in place with a sheetmetal gasket 1½ inches wide, and clinching both collar and gasket to the trap by two bolts fastened on the inside. A sheetmetal cover (not shown in Fig. 1), which closed the hole and thus prevented

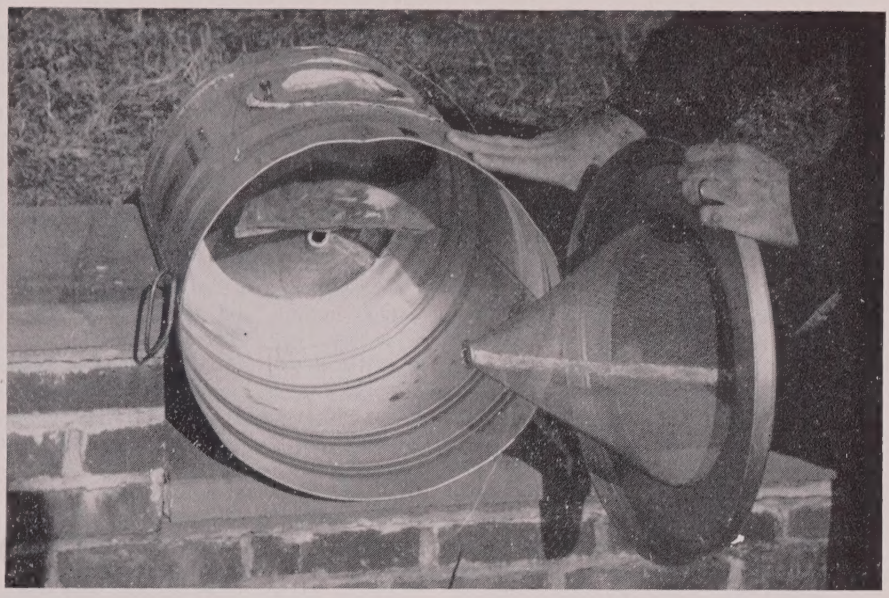

FIG. 1.—Mosquito trap opened at end to show a pigeon in the nylon pocket. The metal cover has been removed from the hole in the top through which the bait is introduced.

mosquitoes from reaching the host animal without entering through the baffles, was secured by two additional bolts. These, unlike the first two, were fastened externally. The underlying purpose of this hatchway was to permit removal of the bait animal to a safe place before anesthetizing the trapped mosquitoes. Four of these traps were used.

Except for the turtles, which were placed unrestrained in the nylon pocket, the host animals were immobilized to reduce their interfering with mosquito feeding. Each bird was placed in the toe of a nylon stocking, which had been cut off just above the heel. The bird's back rested on the sole of the stocking, and its feet were bound by the top of the stocking and secured together by an elastic band. Thus immobilized, the bird was placed on its back in the nylon pocket. In addition to immobilizing the bird, this procedure increased the surface area available to the mosquitoes; an unrestrained bird would stand in the cloth pocket, and its feet would provide very little body area susceptible to mosquito attack. The amphibians tested also were immobilized by using stockings. The mammals and the snakes were restrained by wrapping them in galvanized hardware cloth of ¼-inch mesh. The larger hosts so wrapped (rat, squirrel, rabbit, and snakes) would not fit in the nylon pocket and were placed on the bottom of the mosquito trap. The white-footed mice tended to damage the nylon pocket by gnawing, so they, too, were placed on the bottom of the trap.

The traps, restraining materials, and nylon pockets were washed after each use to remove animal odor. Mammals generally fouled the traps with urine, so a pad of cotton was placed on the floor of the trap to absorb it.

To overcome bias in the mosquito collections which would result from trapping at four different sites, the traps were suspended from the four horizontal arms of a rotary (13) that was turned by an elec-

tric motor at the rate of 0.2 revolution per minute. Each arm of the rotary extended 4 feet from the vertical axis, and each trap was about 5 feet above the ground.

The experimental design used for each test was derived from a randomly formed Latin square (15). The basic design provided for a 4-night test with each of four species of host animal exposed in a different trap on each successive night. Nine 4-night tests were made, each with three or four different species representing from one to three classes of vertebrates. The different species of host and any controls were changed nightly with respect to both position and sequence. Each trap, however, was always hung from the same arm of the rotary. With one exception each test was run from about 4 p.m., E.D.T., to about 8 a.m., E.D.T., on four consecutive nights. Four of the tests included, as a control in place of one host, either an unbaited trap (7 trap-nights) or a trap containing 50 adult *Culex pipiens* (4 trap-nights).

A difference from previous rotary tests of host preference was the attempt to obtain more representative information on a given species of vertebrate by exposing a different individual of the selected species each night. It was not always possible to obtain four species of a given species, however, and some individual hosts were tested from 2 to 6 nights.

RESULTS. Each of the 25 selected vertebrate species was exposed successfully from 1 to 11 times. A few of the birds and mammals, but none of the reptiles or amphibians, either died or became free of the restraining material during the testing. All data from these unsuccessful trials were discarded.

Thirteen different species of mosquitoes were caught in the 113 successful trials of traps baited with vertebrates. There were large totals of four species (7,168 *Culiseta melanura,* 4,450 *Culex salinarius,* 2,030 *C. pipiens,* and 1,198 *Aedes canadensis*), and smaller totals of four others (100 *Culex restuans,* 100 *Mansonia perturbans,* 77 *Culiseta minnesotae,* and 48 *Culiseta morsitans*). The five remaining species were taken in very small numbers. (Five engorged *Aedes abserratus,* one engorged and one unengorged *Aedes excrucians,* and one unengorged *Aedes vexans* were taken in mammal-baited traps; one *A. abserratus,* one *Aedes cantator,* and one *Aedes triseriatus,* all engorged, were taken in bird-baited traps.)

Three species of bird, three of mammal, one of reptile, and three of amphibian were tested on nights when no *C. pipiens* were trapped, and no more tests of these vertebrates were made after *C. pipiens* became abundant. Similarly, various vertebrates were tested on nights when some species of mosquitoes were apparently absent. Therefore, in calculating the average number of a species of mosquito attracted to a given vertebrate, the data have been limited to observations made on those nights when at least one specimen of the mosquito was trapped (even in an unsuccessful trial). This restriction tends to distort somewhat the average attraction in the scarcer mosquitoes by making it proportionately higher than in those species that were common throughout the season.

In addition to average attraction of a given mosquito to a particular vertebrate, there is a second measure of host preference in the percentage of engorgement: total engorged *times* 100 *divided* by total trapped. All or most of the mosquitoes that fed were conspicuously engorged, perhaps because of the general use of restraining materials which favored uninterrupted feeding.

In general, the warm-blooded vertebrates were much more attractive to the mosquitoes than the cold-blooded vertebrates, the birds were more attractive than the mammals, and the amphibians were more attractive than the reptiles (Table 1, Figure 2). The figures for engorgement are certainly affected by the restraint of the test animals but indicate that as a class, the birds were more acceptable as a source of blood than the mammals. In

the cold-blooded vertebrates, which usually attracted very small numbers of mosquitoes, the reptiles were more acceptable than the amphibians. In fact, there was no feeding at all on two of the amphibians, the red-backed salamander and the American toad.

The average number of *C. melanura* attracted to a bird, 153, was the highest for any mosquito on any class of host. The engorgement of *C. melanura* was highest on birds, though only 55 percent. Even so, there were 2,878 specimens which fed on birds, the highest number of any mosquito feeding on any class of host. *C. melanura* was attracted less frequently to mammals and engorged on them at a low rate (only 12 percent). *C. melanura* followed the general pattern of higher attraction to amphibians than reptiles but lower engorgement on amphibians than on reptiles; in fact, this mosquito did not feed at all on amphibians.

The average attraction of *C. pipiens* showed high preference for birds over mammals similar to that of *C. melanura,* and the percentage of engorgement on birds, 85, was the highest rate in any of the four common mosquitoes for any class of vertebrate. Like *C. melanura,* it fed to a limited extent on reptiles, but did not fed at all on the one amphibian (American toad) against which it was tested.

C. salinarius was quite different in its behavior. The average attraction to (76 mosquitoes) and engorgement on (57 percent) birds as a class were not significantly different from mammals as a class (66 and 45, respectively). This mosquito is not, therefore, primarily a bird feeder as are *C. melanura* and *C. pipiens. C. salinarius* also fed to a limited extent on both reptiles and amphibians.

A. canadensis, like *C. salinarius,* was not primarily attracted to birds; the number attracted to mammals was higher than for any other class of vertebrates. The engorgement rates for reptiles, birds, and mammals were high, but the rate for amphibians was low.

To explore the reliability of the figures for average attraction of the four common mosquitoes to the four classes of vertebrates, each separate single-night comparison of two vertebrates of different classes was ranked for each species of mosquito as won, lost, or tied (excluding tied with zero score) according to the total numbers of the mosquitoes in the two traps. The mammal was more attractive than the bird, or equally attractive, 4 out of 21 times in *C. melanura,* 9 out of 21 times in *C. salinarius,* and 14 out of 21 times in *A. canadensis.* (There were only four bird-mammal comparisons in *C. pipiens.*) The amphibian was more attractive than the reptile, or equally attractive, 3 out of 5 times in *C. melanura,* 2 out of 8 times in *C. salinarius,* and 6 out of 8 times in *A. canadensis.* The only other departures from the ranking determined by class averages were 10 out of 52 comparisons in *A. canadensis.* All of these exceptions are seen to correspond very neatly with the decrease in ornithophily from *C. melanura* and *C. pipiens* through *C. salinarius* to *A. canadensis.*

Four mosquitoes were represented by only 48 to 100 specimens (*Culex restuans, Mansonia perturbans, Culiseta minnesotae,* and *C. morsitans*). They were similar in having higher attraction to, and engorgement rates on, warm-blooded than cold-blooded vertebrates (table 2); however, the percentage engorgement of *C. restuans* on mammals (16) was outstandingly low in comparison with that for the other three species (64 to 84).

When the four traps used in all the tests were "baited" with 50 adult *C. pipiens* and operated on successive nights as controls, they contained only 44, 44, 41, and 43 *C. pipiens* at the end of the respective testing periods. In the course of the testing, two *C. melanura* entered one trap and three *C. melanura* another. The fact that the loss of *C. pipiens* from the traps was uniform is considered more important than the escape of a few specimens which were not host-attracted to begin with. The average number of *C. melanura* entering each of these traps (1,

2) may be compared with the average numbers of the mosquitoes entering the unbaited control traps in 7 nights of testing (based on the nights when each species was known to be present: 5 *C. salinarius*, 4 *A. canadensis*, 1 *C. pipiens*, 0.3 *C. melanura*, and 0.2 *C. minnesotae*. The relatively high averages of *C. salinarius* (higher than the average attraction to reptiles) and *A. canadensis* (higher than the average attraction to amphibians) agree with the observation that these

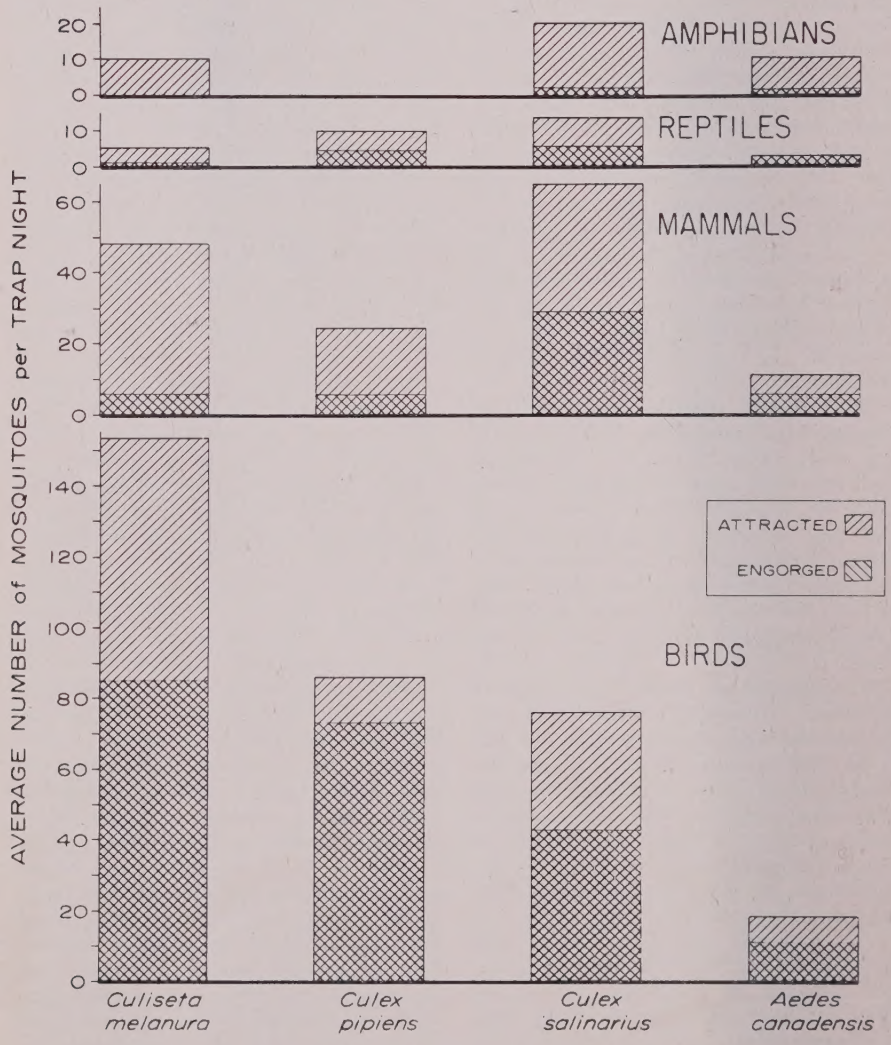

FIG. 2.—Average number of four mosquito species attracted to and engorged on vertebrates per trap night at Raynham, Massachusetts during 1959.

species were more generally attracted to all four classes of vertebrates than were *C. melanura* and *C. pipiens.*

DISCUSSION. The average attraction and engorgement of the four common mosquitoes for the four classes of vertebrates can be discussed with considerable confidence, and the evidence is quite conclusive that *C. melanura* and *C. pipens* were attracted to and fed upon birds much more than any of the other three classes of vertebrates. There were suggestions of significant differences between some vertebrate species within a class (*e.g.,* engorgement rates for chicken vs. starling, and for cottontail vs. vole); however, because of the many variables involved, it is not believed that valid conclusions

TABLE 2.—Attraction and engorgement of four mosquito species in tests with twenty-five vertebrate species conducted at Raynham, Massachusetts during 1959 [1]

	Culex restuans		*Mansonia perturbans*		*Culiseta minnesotae*		*Culiseta morsitans*	
Vertebrate species	A.[2]	E.[3]	A.	E.	A.	E.	A.	E.
Birds								
Catbird	2	0	..	..	..	..	..	..
Chicken, 4–5 week	1	80	2	100	1	100	1	100
Grackle, common	0	0	3	93	1	100	1	100
Pheasant, immature	4	100	2	100	2	100	1	88
Pigeon, domestic	3	42	2	80	2	92	3	91
Sparrow, English	2	0	1	100	4	22	0	0
Starling, immature	0	0	0	0	4	92	2	80
All birds	1.8	70	2.0	96	1.9	81	1.3	89
Mammals								
Bat, little brown	0	0	1	0	0	0	1	0
Chipmunk, eastern	1	0	..	..	2	75	0	0
Mouse, white-footed	2	0	1	100	1	0	..	..
Rabbit, eastern cottontail	2	33	2	77	3	75	1	50
Rabbit, domestic	1	100	2	88	0	0	..	..
Rat, Norway	2	0	3	100	1	0	..	..
Squirrel, eastern gray	0	0	0	0	3	56	1	75
Vole, redback	8	13	1	33	1	100	1	100
All mammals	1.5	16	1.4	84	1.7	64	1.0	64
Reptiles								
Snake, eastern garter	1	0	0	0	1	0	..	..
Snake, eastern milk	0	0	0	0	0	0	0	0
Snake, northern water	1	20	1	100	0	0	1	75
Turtle, eastern box	1	33	0	0	0	0	1	0
Turtle, eastern painted	1	0	0	0	0	0	..	..
Turtle, eastern spotted	.0	0	..	..	1	100	0	0
All reptiles	0.4	22	0.1	100	0.1	50	0.3	60
Amphibians								
Bullfrog	0	0	1	0	0	0	1	0
Frog, green	0	0	2	0	1	0	0	0
Salamander, red-backed	0	0	0	0	1	0	0	0
Toad, American	0	0	0	0	0	0	0	0
All amphibians	0	0	0.4	0	0.2	0	0.1	0

[1] Total number of trap nights when each mosquito was present was: *C. restuans,* 89; *M. perturbans,* 76; *C. minnesotae,* 70; *C. morsitans,* 61.

[2] Average number attracted per trap night when at least one specimen of the species was taken in any trap. Dashes indicate mosquito species absent during all trials with the vertebrate.

[3] Percent of mosquitoes engorged (all trap nights combined). Dashes indicate mosquito species absent during all trials with the vertebrate.

can be made concerning differences between species within any one of the four classes of vertebrates.

No other tests of host preference are known to have involved so many species of mosquitoes, but there is no apparent indication of interference between the different kinds, and in *C. melanura* at least, the percentage found engorged in each trap shows no relation to the total number of other species of mosquitoes.

With regard to the epidemiology of eastern encephalitis, the results not only support the hypothesis that *C. melanura* feeds primarily on birds, but show that, because it feeds also on mammals, it must be considered a potential endemic as well as enzootic vector of EE virus. Moreover, though it did not feed upon amphibians, it did engorge upon reptiles and thus could be involved in the overwinter transmission cycle if, as recently suggested for western encephalitis virus (16, 17), snakes were indeed the winter reservoir.

C. pipiens and *A. canadensis* have not yet been shown to be involved in the transmission of EE. Laboratory tests indicate that *C. salinarius* has a poor vector potential for EE virus (18), but engorged specimens collected from swamps have been found to contain EE virus, which could have been in the recently ingested blood rather than in the mosquito tissues (7). Though *C. salinarius* might have little importance as an interepidemic vector, its tendency to feed on mammals as well as birds would give it great potential importance during an epidemic season even if only as a mechanical transmitter.

C. restuans and *M. perturbans* have been found naturally infected with EE. The limited amount of data on these two species at least indicates that they feed on both birds and mammals and to some extent on reptiles.

SUMMARY. Seven species of birds, eight of mammals, six of reptiles, and four of amphibians were exposed as bait in mosquito traps in southeastern Massachusetts to ascertain the species and numbers of mosquitoes which would be attracted to and feed upon each type of animal. Four mosquito species (*Culiseta melanura, Culex pipiens, Culex salinarius,* and *Aedes canadensis*) were trapped in sufficient numbers to permit broad classification of the mosquito-host relationships. All were attracted to and fed to a limited extent on reptiles. All but *C. pipiens* also were attracted to amphibians, but only *C. salinarius* and *A. canadensis* fed upon them. Both *C. melanura* and *C. pipiens* were attracted to and fed upon birds much more than mammals, but *C. salinarius* and *A. canadensis* showed no significant difference between birds and mammals. Two other mosquitoes that have been reported naturally infected with EE, *Mansonia perturbans* and *Culex restuans,* were trapped in small numbers only. Both were attracted to warm-blooded more than to cold-blooded vertebrates, and both fed, at least to some extent, on all classes of vertebrates except amphibians.

The data support the hypothesis that *C. melanura* is the primary enzootic vector of eastern encephalitis, maintaining the summer reservoir in wild birds by transmitting the virus from one bird to another. It also could be a vector in summer or winter transmission cycles involving wild mammals or reptiles.

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THE EFFECT OF PHENYLTHIOUREA AND 4-CHLORORESORCINOL ON *AEDES AEGYPTI* LARVAE

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Introduction. During the course of experiments with sub-lethal concentrations of compounds that inhibit various enzyme systems in insects, two chemicals known to block tyrosinase were tested in larval medium of *Aedes aegypti*. Phenylthiourea and 4-chlororesorcinol were reported by Kull, *et al.* (1), to be effective in inhibiting melanin formation in the regenerating fin of platy fish during *in-vivo* experiments. However, while little toxicity to the fish was indicated, no observations of post-treatment effects on growth and reproduction were made. For this purpose it was postulated by the author that other aquatic animals, such as rapidly growing mosquito larvae, would be excellent tools for tests with enzyme-blocking compounds. Consequently this study using low concentrations of these compounds was conducted, utilizing *Aedes aegypti* larvae as test animals. The object of this paper is to report the *in-vivo* inhibition of melanin formation and the growth-retarding effect of these chemicals.

Methods. A series of solutions of 4-chlororesorcinol (M. W. 144.56, Eastman

organic chemicals) and 1-phenyl-2-thiourea (M. W. 152.22, Eastman organic chemicals) was established so that concentrations of each chemical were obtained as follows: 0, .1, .01, .001, .0001, 00001 M. Two hundred ml. of each concentration of the two solutions were placed in flasks with .1 gm. of Purina rabbit feed pellet and 50 second-stage larvae of *Aedes aegypti,* and maintained for observation at room temperature (70° ± 4° F.). For the first four days larval mortality was recorded each 24 hours, and pupae and adults that developed in the low concentrations of the compound were harvested for further study. Adult mosquitoes were transferred to lantern chimney cages, given sucrose solution for food, allowed a three-day mating period; and then the females were given a blood feeding. These mosquitoes were maintained until egg deposition, the eggs then conditioned and tested for hatchability. The procedure was then repeated twice for replication, and the results summarized and compared to those obtained from controls.

RESULTS. The higher concentrations of both chemicals in the larval medium (.1, .01 M) were insecticidal, killing all of the larvae within four days. Larval mortality in both chemical solutions at .001M concentrations was quite variable, ranging from 3 to 88 percent among the six replicates. Larvae surviving in this concentration and in the .0001 M and .00001 M concentrations of both phenylthiourea and the 4-chlororesorcinol exhibited signs of lack of melanin formation. They also remained semi-transparent and failed to develop the brown cuticular coloration of the control larvae.

A more pronounced effect was observed in the growth of the larvae. The developmental period was extended to 15–21 days as compared to 7–10 days to pupation of the control larvae. There was considerably more pupal mortality in the treated groups than in the controls. This amounted to as much as 40 percent in those reared in .001 M. 4-chlororesorcinol. Adult mosquitoes from larvae reared in this solution failed to live more than 10 days. Only 4 females of a total of 83 adult mosquitoes that developed after rearing in the 4-chlororesorcinol solution lived beyond 5 days and developed eggs. These eggs, however, after oviposition, conditioning and testing for hatchability, proved to be viable and produced vigorous larvae. Mortality in the pupal and adult stages of mosquitoes from larvae grown in concentrations below .0001M phenylthiourea was not exceptionally more than among controls. Phenylthiourea treatment of larvae likewise did not affect the fertility or longevity of the adult mosquitoes.

Pupae and adult mosquitoes, developing from larvae that exhibited inhibition of melanin formation during the larval stage retained normal coloring in the pupal and adult stages so that it was impossible to distinguish a difference between those and treated or control groups.

DISCUSSION. In the experiments with 4-chlororesorcinol of Kull, *et al.* (1) there was the complete inhibition of melanin in regenerating fin of one species of fish. However, in another species of the same genus, the melanin inhibition was not complete—suggesting that even in closely related fish the pigment producing cells converted tyrosine to melanin under different conditions. In their experiments, when the treated fish were transferred from medicated to untreated water, the melanin inhibition was reversible and the regenerating tissue became black. However, there was no extended investigation of the post-treatment effects. These effects on survival, growth and reproduction are of considerable interest inasmuch as little is known of the activity of tyrosine in the reproduction and longevity of insects. Mosquitoes, particularly males that feed only on carbohydrates in the adult stage, theoretically accumulate the protein requirements for reproduction only during their larval life in the aquatic medium. This, together with their short generation time, makes them exceptionally good

experimental tools for the investigation of enzyme-inhibiting compounds *in vivo*.

While tyrosine is an amino acid that is not generally considered essential for growth of insects (Gilmore (3)), the addition of two compounds of known anti-tyrosinase activity at sub-insecticidal concentrations more than doubles the time necessary for development to the pupal stage. Likewise, the increased pupal mortality and greatly reduced longevity of adults following larval treatment with 4-chlororesorcinol indicate either a carry-over of toxicity or the induction of a severe nutritional deficiency.

As pointed out by Brown (4) phenylthiourea is of considerable interest in that it is selectively insecticidal against DDT-resistant flies. Chemicals with a similar mode of action that are more insecticidal then phenylthiourea may be of considerable importance if they are also selectively effective against DDT resistant insects. While 4-chlororesorcinol did not affect the fertility of *A. aegypti*, it is worthwhile to note that solutions of this compound are larvicidal at concentrations that are non-toxic to fish; therefore, studies utilizing DDT-resistant larvae are suggested.

Regardless of their larvicidal potential, chemicals that effectively lengthen the larval growth stage in the aquatic medium may be useful in enhancing biological control. The longer mosquito larvae are in contact with parasitic fungi in the water and are exposed to predatory fish, the better chance these agents have to produce an effect on the larval population; 4-chlororesorcinol may be useful for this purpose since it significantly lengthens larval developmental time at dilutions that are sub-toxic to fish. It has the additional advantage of rapid dissociation in aqueous solution; so the possibility of providing a cumulative residual toxicant to endanger wildlife is minimal.

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THE DISPERSAL OF MOSQUITOES BY RAILROAD TRAINS INVOLVED IN INTERNATIONAL TRAFFIC

E. G. CAMPOS,[1] H. A. TREVINO,[1] AND L. G. STROM [2]

The Quarantine Division of the United States Public Health Service has maintained entomological surveillance of international points of entry into the United States since the early thirties. Principal objectives have been the formulation and implementation of measures to prevent the introduction of arthropods of known or suspected public health importance into this country by international carriers. Mosquitoes have received particular attention. Light traps are operated, natural daytime resting places examined, and larval surveys conducted in strategic areas, in an effort to detect and control exotic vector species which enter by natural means or by improperly disinfested carriers. This surveillance is also concerned with the detection and eradication of such known vectors as *Aedes aegypti* around international entry points, which might become infected from incoming travelers.

Entomological inspections have been primarily concerned with aircraft and surface vessels, since they are responsible for the bulk of international taffic. The value of this program has been demonstrated by numerous exotic arthropod interceptions, as reported by Hughes (1949) and Hughes and Porter (1956). The role played by other modes of international carriers such as trains and automotive vehicles in the dispersal of insects has not been so well clarified.

Brownsville, Texas, is of considerable quarantine importance, with international entry possible by aircraft, surface vessel, train or by automobile and truck. A mild climate and abundant surface water provide a situation favorable to the establishment of imported mosquitoes. The large and diverse local mosquito fauna makes the discovery of newly-arrived species difficult.

An effort has been made to evaluate the importance of railroad trains in transporting mosquitoes and other insects of public health significance. In February 1958, sporadic inspections of railroad trains were begun on their arrival at Matamoros, Mexico, from the interior of that country, and prior to their admission to the United States. Matamoros, Mexico, is situated on the Rio Grande River, directly across from Brownsville. One train arrives daily with varied products, particularly from the States of Neuvo Leon, Durango, Tamaulipas, Oaxaca, Morelos, San Luis Potosi, Chihuahua, Coahuila and Veracruz. Trains from Mexico also enter the United States at Laredo, Eagle Pass, Presidio and El Paso in Texas.

A primary interest in inspecting the railroad cars in Matamoros has been the search for *Aedes aegypti*. Recent surveys for this yellow fever vector by personnel of the Foreign Quarantine Division, Public Health Service, have failed to demonstrate its presence in and immediately adjacent to international points of entry along the Texas-Mexico border.

The trains in question arrive in Matamoros during the night and are held until the following morning for inspection of the cars and contents by U. S. Department of Agriculture entomologists. Upon being cleared, the trains enter the United States, with the Port of Brownsville their usual destination. Our search for mosquitoes has been in cooperation with Department of Agriculture entomologists.

The mosquitoes have been taken with aspirators or small sweep nets. As the

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box cars are dark, a strong flashlight has always been necessary. Few mosquito recoveries were made from cars of pineapple, bananas or baled cotton, as they are loaded to the roof and difficult to search adequately. Cars of lead bars also produced few mosquitoes, even though they could be examined carefully. A large percentage of the mosquitoes reported on in this study were taken in cars loaded from one-third to one-half full of barite or fluorspar ore. This may well have been due to the fact that these materials are light in color and provide a background against which the mosquitoes can be seen easily. When mosquitoes were found in a car, the point of origin of the latter was ascertained and recorded for reference, if needed.

During the period of Feb. 1958 thru Dec. 1960, 4,436 box cars in 113 trains were inspected for mosquitoes and 3,162 adults were collected alive. Of the total, 98.7 percent were *Culex quinquefasciatus*. Other species taken included *Aedes vexans, Anopheles quadrimaculatus, Culiseta inornata, Anopheles crucians, Mansonia titillans, Uranotaenia lowii, Culex erraticus, Culex tarsalis,* and *Psorophora confinnis* (Table 1.). The *Culex quinquefasciatus* determinations were confirmed by a study of genitalia slides of the males which were taken.

The numbers of mosquitoes caught have little quantitative significance, since only samples were taken from the box cars harboring many specimens. However, these data indicate there is considerable movement of certain mosquito species from the interior of Mexico into the United States in railroad box cars. No *Aedes aegypti* or species not already established in the Brownsville area have been collected.

The transmission of *Culex quinquefasciatus* into the Rio Grande Valley from Mexico is of possible public health significance. The frequency with which St. Louis encephalitis virus has been isolated from the species, in the southern United States, together with population studies, indicates it is an important vector. St. Louis encephalitis virus was obtained from two pools of *C. quinquefasciatus* collected in the early fall months during the 1954 epidemic in Hidalgo County and a single isolation was accomplished the following January (Sullivan, *et al.,* 1957). Wiseman, *et al.,* (1959) reported eight isolations from pooled *C. quinquefasciatus* during the 1957 outbreak in Cameron County. Although Texas State Department of Health entomologists and virologists have made regular collections of mosquitoes in the Rio Grande Valley as part of state-wide encephalitis studies, with the single exception noted above, no virus isolations have been obtained from mosquitoes during inter-epidemic periods. Thus, the possibility exists that

TABLE 1.—Mosquitoes taken from railroad box cars entering the United States from Mexico during the period from February, 1958 through December, 1960

Species	1958 2,106 rr cars	1959 1,577 rr cars	1960 753 rr cars	Total
Culex quinquefasciatus	586	2,034	501	3,121
Aedes vexans	10	1	3	14
Anopheles quadrimaculatus	4	5	5	14
Culiseta inornata	0	0	4	4
Anopheles crucians	0	2	0	2
Mansonia titillans	0	2	0	2
Uranotaenia lowii	0	2	0	2
Culex erraticus	0	0	1	1
Culex tarsalis	0	1	0	1
Psorophora confinnis	1	0	0	1
Total	601	2,047	514	3,162

the encephalitis viruses are introduced into the area by infected arthropods or vertebrates, and do not exist in an endemic state. Several of the larger pools of *C. quinquefasciatus* collected from railroad box cars in 1960 have been shipped to the Texas State Department of Health Laboratory for virus studies, with negative results.

Anopheles quadrimaculatus were taken in numbers sufficient to indicate that there is some international dispersal of the species by railroad box cars. Even though there has been a decline to the point of disappearance of malaria in the United States and much of Mexico, sporadic cases continue to occur. Two cases were brought to our attention in Cameron County in September, 1960. The malaria potential is of concern to public health workers, especially in areas such as the Lower Rio Grande Valley of Texas, with large populations of *A. quadrimaculatus.*

Of interest was the recovery of but a single *Culex tarsalis.* The species is frequently intercepted on aircraft arriving in Brownsville from Mexico.

SUMMARY. Inspections of railroad box cars have been conducted upon their arrival in Matamoros from the interior of Mexico, just prior to their admittance into the United States. From February 1958, through December 31, 1960, 4,436 cars in 113 trains were examined and 3,162 adult mosquitoes were collected alive. They were primarily *Culex quinquefasciatus,* with nine other species represented by small numbers.

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BIOCHEMICAL STUDY OF A MALATHION-TOLERANT STRAIN OF *AEDES AEGYPTI*

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A malathion-tolerant strain has been developed by larval selection with malathion applied to a colony of *Aedes aegypti* from Penang, Malaya (Brown and Abedi, 1960). This strain attained a 5-fold increase in malathion-tolerance, but simultaneously acquired a cross-resistance to DDT and DDD in excess of 30 times the normal.

Malathion-resistance, as first studied in the house fly, has been found to involve an increase in the detoxification of malaoxon (March, 1959), and a concomitant decrease in the enzyme aliesterase (Oppenoorth, 1959). Later, the strong and specific malathion-resistance developed in a strain of the mosquito *Culex tarsalis* has been found to involve a marked increase in carboxyesterase activity detoxifying malathion, and a smaller increase in phosphatase activity detoxifying malaoxon (Matsumura and Brown, 1961). The aim of this study was to ascertain whether these or other biochemical mechanisms were associated with the malathion-tolerance of the Penang strain of *Aedes aegypti.*

The malathion-tolerant Penang strain

was tested in the F_{11} generation, after having been maintained without selection pressure since the F_8 generation, the last to be reported by Brown and Abedi (1960). The LC_{50} for the F_{11} was 0.65 p.p.m. malathion, as compared with 0.31 for a susceptible Guelph strain. The LC_{50} level to malaoxon was 4.9 p.p.m., as compared with 2.6 p.p.m. for the normal strain, and 1.3 p.p.m. for the susceptible Guelph strain. Thus the malathion-tolerant Penang strain in the F_{11} was twice as tolerant as the normal to both malathion and malaoxon.*

The aliesterase activity was assessed according to the method of Oppenoorth (1959), using methyl n-butyrate as the substrate. Measured as the cu. mm. CO_2 produced per gm. tissue per minute, the activity proved to be 11.1 ± 2.8 for the malathion-tolerant strain, and 8.6 ± 3.2 for the normal strain. This difference is without statistical significance, the t_7 being 0.59.

The phosphatase activity and carboxyesterase activity in homogenates of either strain were assessed *in vitro* according to the method of Matsumura & Brown (1961), using radioactive malathion as the substrate. The results with the three strains (Table 1) indicate the malathion-tolerant and the normal strains showed virtually identical activities in these two detoxifying enzymes; both Penang strains showed slight differences from the Guelph strain in the enzyme activities, but they were not quite significant statistically.

Since no difference in detoxifying activity was evident, an experiment was performed to investigate the absorption of malathion by larvae of the different strains *in vivo*. Groups of 25 larvae were exposed to 1 p.p.m. radioactive malathion in 25 ml. water for 1 hour; they were then removed to 25 ml. distilled water for 1 hour, and the radioactivity remaining in the larvae and excreted into the water was assessed according to the method described by Matsumura and Brown (1961). The results (Table 2) show that all strains ingested similar amounts of malathion, but they also reveal that the malathion-tolerant Penang strain excreted more and retained much less of the insecticide within the body. In fact, the amount actually retained (and thus absorbed internally) in the malathion-tolerant strain was less than one-twelfth as much as in the other two strains. The difference was highly significant statistically, the t_7 values being respectively 3.48 and 3.97.

Since the malathion-tolerant strain was characterized by having also gained a high cross-resistance to DDT, the strains were similarly compared for their absorption and retention after exposure to 1 p.p.m. radioactive DDT. It was found that the malathion-tolerant strain retained (absorbed internally) only one-quarter as much DDT as the normal Penang strain. The lower absorption in the malathion-tolerant strain was highly

TABLE 1.—Phosphatase and carboxyesterase activities in malathion-tolerant and susceptible strains of *Aedes aegypti*

Strain	Phosphatase products		Carboxyesterase products	
	Percent*	t_0**	Percent*	t_0**
Malathion-tolerant Penang	10.0±1.3	0.42	2.5±0.2	0.71
Normal Penang	9.3±0.9	..	2.3±0.2	..
Susceptible Guelph	6.2±1.3	1.96	2.8±0.4	1.11

* Percentage of malathion radioactivity converted to hydrolytic products.
** Statistical comparison with normal Penang strain.

* This strain gradually reverts toward susceptibility when maintained without selection pressure.

TABLE 2.—Malathion and metabolites excreted and retained internally: micrograms per 100 larvae

Strain	Excreted into clean water			Retained in the body
	Malathion (+ malaoxon)	Phosphatase products	Carboxyesterase products	
Malathion-tolerant Penang	1.50±.77	5.81±1.45	1.75±.79	0.14±.13
Normal Penang	2.00±.72	2.93± .23	0.11±.11	1.77±.47
Susceptible Guelph	0.75±.52	3.46±1.12	0.88±.52	2.12±.52

significant statistically not only at 1 p.p.m. ($t_5 = 3.50$) but also on exposure to 5 p.p.m. DDT ($t_5 = 5.05$).

It is therefore evident that the malathion-tolerance of the Penang strain of *Aedes aegypti,* in contrast to the malathion-resistance studied in *Culex tarsalis,* derives not from increased detoxification but from decreased absorption of malathion. This decreased absorption is even more marked in the case of DDT, to which the cross-resistance is much greater than to malathion itself. Therein the malathion-tolerant strain resembles a DDT-resistant strain developed in the Penang stock by DDT pressure, which was found to absorb and retain only one-third as much DDT as the normal strain.

SUMMARY. Larvae of a malathion-tolerant strain of *Aedes aegypti,* developed by laboratory selection of stock from Penang, Malaya, were found not to differ from the normal in activity of the detoxifying enzymes phosphatase or carboxyesterase, nor in aliesterase. They did differ in showing only one-twelfth as much absorption and retention of malathion as larvae of the normal strain. Moreover, the high cross-resistance shown by this strain to DDT was accompanied by only one-quarter as much absorption and retention of DDT as the normal.

The work was performed on grants-in-aid from the World Health Organization and the National Research Council of Canada. Assessments of DDT absorption were kindly made by Mr. P. G. Fast.

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NOTES ON THE BIONOMICS AND SEASONAL OCCURRENCE OF MOSQUITOES IN SOUTHWESTERN GEORGIA

G. J. LOVE and M. H. GOODWIN, Jr.[1]

Introduction. Observations on the abundance and distribution of mosquitoes in southwestern Georgia have been made since 1939 in connection with studies on the natural history of arthropod-borne diseases. Results of most studies and observations have been reported separately (see bibliography) but much material which was collected incidentally or resulted from preliminary experiments has not been made available. The purpose of this paper is to record pertinent unpublished observations made over an eighteen year period, 1939 to 1957. Some material is fragmentary and some of the comments concerning bionomics of various species are speculative. It is believed, however, that the data presented are of interest and value to culicidologists.

In 1951, an extensive, long-range study on the microhabitat of mosquitoes was inaugurated (Platt, 1955). The present report does not give details of this recent work since it is developing rapidly and progress to date has been reported adequately. (Platt, 1957; Platt *et al.*, 1957; Platt *et al.*, 1958; Love *et al.*, 1958).

Topography And Climate of Study Area. All observations were made in the experimental area of the Emory University Field Station. The topography and climate were described in detail by Hendricks and Goodwin (1952a). The area covers approximately 200 square miles of Baker and Early Counties, Georgia. The center of the area is approximately latitude 31° 20′ N., longitude 84° 20′ W., about 190 miles west of the Atlantic Ocean and 90 miles north of the Gulf of Mexico. The elevation ranges from 100 to 250 feet above sea level. The area lies within the Dougherty Plain which covers about 7,000 square miles. Limestone, which once overlay the area, has been removed by the solution of ground water leaving a residuum of sands and clays ranging in thickness from 70 to 100 feet. Beneath the residuum are undissolved remnants of Oligocene and Eocene limestones. Numerous sinks and surface depressions occur in the area. It is believed that these sinks result from the collapse of the overburden when the deep-lying limestone is dissolved. Depressions thus formed have gentle sloping sides and lack evidence of fracture of the surface strata. Limestone sinks, which are caused by solution at or near the surface, have steeper sides and show evidence of slumping of the surface strata. Formation of the lime-sink ponds was described by Goodwin and Lenert (1943).

The climate of the study area, in common with that of the rest of the region, is warm and moist. Normal annual precipitation is about 50 inches. The heaviest precipitation occurs ordinarily during July and August and a secondary peak usually is reached during February and March. The fall season is the driest of the year. Wide variations in amounts of annual rainfall are experienced.

For the years 1892 to 1957, annual rainfall in the southern division of Georgia ranged from 28.97 to 66.53 inches (U. S. Weather Bureau, 1958). The July precipitation ranged from 3.56 to 14.69 inches. Average monthly precipitation figures in inches for three stations near the area having 50 years or more of records are shown in Table 1.

The average annual temperature at Bainbridge, located about 24 miles south of the center of the area, is 67.7° F. (Table 2). Very changeable temperature conditions prevail in winter; daily maxima

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TABLE 1.—Average monthly precipitation in inches at Albany, Blakely, and Bainbridge for period 1882–1957

Month	Albany[1]	Blakely[2]	Bainbridge[3]
January	3.90	4.44	3.80
February	4.59	4.96	4.33
March	5.01	5.54	4.68
April	4.00	4.51	4.18
May	3.81	3.63	3.28
June	4.31	4.50	4.76
July	5.96	6.82	6.64
August	5.36	5.77	5.66
September	3.52	3.97	4.41
October	2.26	2.56	2.19
November	2.50	3.08	2.37
December	3.90	4.44	4.30
Annual	49.12	54.22	50.60

[1] Twenty-one miles N. E. from center of study area.
[2] Eighteen miles W. from center of study area.
[3] Twenty-four miles S. from center of study area.

TABLE 2.—Mean monthly air temperature in degrees Farenheit at Bainbridge, Georgia, based on records for 1882–1957

January	52.7
February	54.8
March	61.0
April	67.5
May	74.9
June	80.6
July	81.7
August	81.5
September	78.1
October	68.3
November	58.3
December	52.7
Annual	67.7

are rarely below 32° F., and frequent warm periods occur. The highest recorded January mean temperature exceeds the lowest by about 20° F. Summer temperatures are more consistent. The highest recorded July mean temperature exceeds the lowest by about 6° F. The average date of the first killing frost is November 15 and of the last is March 10. The average length of the growing season is 250 days. Snowfall is rare.

Relative humidity is highest in winter, and a secondary peak occurs in midsummer. Spring and fall have the driest atmosphere, conforming to the pattern of rainfall distribution. Evaporation from a U. S. Weather Bureau Class A pan at the Emory University Field Station averaged 55.74 inches annually for 12 years (1946–1957).

METHODS OF COLLECTING MOSQUITOES. From 1939 to 1941 adult mosquitoes were collected only from houses, barns, culverts, hollow trees, and similar natural resting places. In 1941 an artificial resting station, primarily for the study of *Anopheles quadrimaculatus,* was developed by Goodwin (1942). These stations, one-foot cubical boxes with one end open and painted red inside and out, provided a comparatively uniform resting place which could be located near breeding areas and in other situations where mosquitoes congregate. Also in 1941, use of battery and power-line operated New Jersey type light traps was commenced. Beginning in 1952, mechanical sweep nets were employed. These traps used no attractant and thus had advantages over other traps in sampling all species active within range.

During the winters of 1944–45 and 1945–46, collections of *Anopheles* spp. and some culicine species were made by fumigating hollow trees on days following nights during which the temperature fell below freezing (Zukel, 1949a, 1949b).

Mosquito larvae were usually collected from ponds and other large areas by skimming the water surface with an enamel pan or dipper. Larvae were collected from tree holes with a siphon. In cases where larvae could not be removed directly from artificial containers, because of small orifice or presence of debris, most of the water was decanted and the remainder, containing the larvae, was poured into a shallow white pan.

Mosquito collections from 1938 to 1948 were made primarily in connection with studies of malaria and anophelines. During this period no regular effort was made to collect all species in the area. A sufficient number of collections was made over the nine-year period, however, to obtain tenable data on seasonal distributions of prominent species. After 1948, collec-

Fig. 1.—Monthly mean elevation of water surface in Mossy Pond, Baker County, Georgia and monthly precipitation, 1945–1954. This pond was dry during 1955, 1956 and 1957.

tions were designed to obtain all culicine species.

The nomenclature used in this report is based on Pratt's Check List of North American Mosquitoes (1956).

DESCRIPTION OF BREEDING AREAS. The mosquito breeding places in southwestern Georgia consist of "permanent" ponds, temporary pools, roadside ditches, tree holes, and many types of artificial containers. With the exception of tree holes and artificial containers, the character of most breeding areas is determined by topography and by climate, particularly precipitation and temperature. The character of a particular breeding area and the fauna supported, may vary from year to year.

An example of variations in climatic factors and the associated variations in a breeding area is shown by the monthly precipitation data and water level fluctuations at Mossy Pond (Figure 1). This pond is representative of the "permanent" limesink ponds, the most important habitat for mosquitoes in the area. From 1939 until 1949 Mossy Pond held water continuously (Figure 2) and the mosquitoes produced were characteristic of the fauna in relatively permanent, mature limesink ponds. *Anopheles crucians* Wied., *A. quadrimaculatus* Say, *Culex erraticus* (D. and K.), *Uranotaenia sapphirina* (O. S.), and *Mansonia perturbans* (Walk.) were common during the summer months. In the cooler portions of the year *A. crucians, A. punctipennis* (Say), and *C. territans* Walk. were abundant (Table 3). In these years, the water level in the pond receded during the summer (growing season) and rose during winter (water-storage period).

FIG. 2.—Permanent limesink breeding area, Baker County, Georgia

TABLE 3.—Species of mosquito larvae collected from various types of breeding areas in southwestern Georgia

Permanent	Temporary	Intermittent	Tree holes	Artificial containers
A. crucians *	*A. crucians*	*A. punctipennis*	*A. barberi* *	*A. crucians*
A. georgianus *	*A. punctipennis*	*C. pilosus* *	*O. signifera* *	*A. punctipennis*
A. punctipennis	*C. erraticus*	*C. restuans* *	*T. r. septentrionalis* *	*C. p. quinquefasciatus* *
A. quadrimaculatus	*C. peccator* *	*Ae. atlanticus* *	*Ae. triseriatus* *	*C. restuans*
A. walkeri *	*C. territans*	*Ae. canadensis* *		*C. territans*
Culex erraticus *	*Ae. atlanticus*	*Ae. dupreei* *		*O. signifera*
C. nigripalpus *	*Ae. canadensis*	*Ae. infirmatus* *		*T. r. septentrionalis*
C. territans *	*Ae. cinereus* *	*Ae. mitchellae* *		*Ae. triseriatus*
Culiseta inornata *	*Ae. dupreei*	*Ae. sticticus* *		
C. melanura *	*Ae. fulvus-pallens* *	*Ae. thibaulti* *		
M. perturbans *	*Ae. infirmatus*	*Ae. tormentor* *		
U. sapphirina *	*Ae. mitchellae*	*Ae. vexans* *		
U. lowii *	*Ae. vexans*	*P. ciliata* *		
	P. ciliata	*P. confinnis* *		
	P. confinnis	*P. cyanescens* *		
	P. discolor	*P. discolor* *		
	U. sapphirina	*P. ferox* *		
	U. lowii	*P. horrida* *		
		P. howardii		
		P. varipes		

* Indicated type breeding area in which the species most commonly occurs.

Fig. 3.—Typical limesink breeding area following several years of drought

In the fall of 1949 and spring of 1950 precipitation was insufficient to restore the water level to the normal spring stage and the usual depletion during the summer further lowered the level. In the winter of 1950–51 rainfall was again deficient and the pond became completely dry late in the summer of 1951 (Figure 3). During 1951 and most of 1952 the mosquito fauna was completely different. The prominent species were *Aedes vexans* (Meig.), *Ae. atlanticus* D. and K., *Ae. infirmatus* D. and K., *Psorophora ferox* (Humb.), *P. confinnis* (L.-A.), *P. ciliata* (Fab.), *P. cyanescens* (Coq.), and during the cooler periods of the year *Aedes mitchellae* (Dyar), *Ae. canadensis* (Theob.), and *Culex restuans* Theob. In the latter part of 1953, the pond level was higher than during any fall month since 1949. Rainfall was greatly deficient in the first months of 1954, the water level dropped during the growing season, and by July the pond was again dry. In 1954, species which develop in "permanent" ponds were produced from January until June; afterward species associated with temporary pools were present.

The substrata of temporary ponds and pools were highly porous. The length of time that water was retained depended upon increment and condition of the water table. During years with normal or heavy precipitation many of the ponds (Figure 4) contained mosquitoes characteristic of permanent ponds; e.g. *U. sapphirina, C. nigripalpus* Theob., and *C. erraticus. Culex peccator* D. and K. was often found in ponds of this type. During periods of extreme drought temporary pools frequently produced few or no mosquitoes since intermittent rains were not

FIG. 4.—Intermittent pond, Baker County, Georgia

sufficient to maintain water in the pools long enough for larvae of *Aedes* and *Psorophora* to mature even though eggs hatched.

Psorophora spp. were the characteristic species found in roadside ditches (Figure 5); *Aedes* spp. and *Culex pilosus* (D. and K.) occurred less prominently in these situations. In common with other types of highly intermittent aquatic habitats, the ephemeral biota in ditches was not consistently characteristic. The mosquitoes found most frequently in roadside ditches occurred also in temporary pools and in "permanent" ponds depending upon the extent of drought.

Artificial containers (Figure 6) produced primarily *Culex pipiens quinquefasciatus* Say in the summer and *C. restuans* in the winter. *Toxorhynchites rutilus septentrionalis* (D. and K.) and *Orthopodomyia signifera* (Coq.) larvae were found throughout the year in artificial containers which held water contaminated with organic materials.

Tree holes (Figure 7) produced *Aedes*

Fig. 5.—Roadside ditch

Fig. 6.—Artificial container

FIG. 7.—Tree hole from which numerous *Aedes triseriatus* larvae were obtained, Baker County, Georgia

triseriatus Say, *Anopheles barberi* Coq., *O. signifera,* and *T. r. septentrionalis.*

SEASONAL DISTRIBUTION OF MOSQUITOES. The collecting techniques used for these studies do not provide an accurate, comparable, quantitative measurement of relative abundance of all species. For example, *A. quadrimaculatus* were taken in large numbers from hollow trees and artificial resting stations, but the numbers of *A. crucians* in these same places were too small to indicate population trends. Con-

versely, light traps in the same areas collected many *A. crucians* but fewer *A. quadrimaculatus*. Comparative data on abundance are necessarily limited to a single species or to populations which are closely related ecologically. Comparison of trends in populations is more reliable, however. For example, the direction and magnitude of variations in populations of adult *A. quadrimaculatus* was the same whether measured by light trap collections or by enumeration of mosquitoes in artificial resting stations. For other species, however, e.g. *Culiseta melanura, Aedes triseriatus,* or *Psorophora ferox,* insignificant numbers of adults were obtained by light trap collections or by other repetitive techniques, to provide comparative data on abundance.

Bait traps using animals or chemical attractants were singularly unsuccessful in collecting adult mosquitoes in the area studied. Bait traps, as well as light traps, measure taxis rather than comparative abundance and hence have limited usefulness in population measurements.

Probably the most accurate information on the relative abundance of various species was obtained from the mechanical sweep nets. Because of the small number of some species collected each night, however, it may be necessary to use this technique for several months or years to obtain valid information. By comparing collections made with light traps with those made with the sweep nets preliminary indices of attractiveness to light were derived for most of the species which occur in the area (Love and Smith, 1957).

Larval collection methods employed in this study were selective for certain species. *Anopheles* spp. are easily collected by skimming the water surface with a pan; but some other species, e.g. *Culiseta melanura* (Coq.), *Psorophora* spp. and *Aedes dupreei* (Coq.), dart to the bottom at the slightest movement of the water surface and remain submerged for several minutes. Consequently, larval collections in this study did not provide tenable data on relative abundance of aquatic stages.

These limitations of both larval and adult collections are emphasized to indicate the reservations held in interpreting the following data which were collected as collateral observation rather than as studies designed specifically to provide comparable information on populations.

Of the 51 species of mosquitoes previously reported from Georgia (Table 4), 42 were collected in the southwestern section of the state during the present study. Four of the remaining nine species, *Anopheles atropos* D. and K., *A. bradleyi* King, *Aedes sollicitans* (Walk.), and *Ae. taeniorhynchus* (Wied.), breed in salt marshes, a habitat which does not occur in the area observed. *Ae. trivittatus* (Coq.) was reported from Lee County, Georgia, 35 miles northwest of the area, by Root in 1924 but has not been reported since. Two female mosquitoes collected at Camp Wheeler, Georgia, in 1943 and identified as *Culex tarsalis* Coq. (Middlekauff and Carpenter, 1944) are the only state record for this species. *Culex pipiens pipiens* Linn. has been identified from collections in the northern part of the state but not from the southern section. *Orthopodomyia alba* Baker was identified from Georgia material in 1945 (Carpenter, *et al.,* 1946) and *Toxorhynchites rutilus rutilus* (Coq.) was reported from Georgia before 1920 (Howard, *et al.,* 1912–17). Neither of the latter species was found in southwestern Georgia during this study.

A summary of records on mosquito collection for the period 1939 through 1957 is shown in Table 5. Data are summarized by months; the species collected and the methods used are shown. An indication of abundance also may be obtained from this table since, in most instances, the species present in greatest numbers were collected by more than one method. *Aedes* was most abundant from March through June (Table 5), *Psorophora* from June through October, and *Culex* from July through November. In the field, succession of *Aedes* and *Psorophora* was especially noticeable. Species of both genera occurred in the same larval habitats, but only a few *P. ferox* and *P.*

TABLE 4.—Species of mosquitoes reported from the state of Georgia, and those collected during the present study in southwestern Georgia

Anopheles atropos [1]	*Aedes atlanticus*
barberi	*canadensis*
bradleyi [1]	*cinereus*
crucians	*dupreei*
georgianus	*fulvus-pallens*
punctipennis	*infirmatus*
quadrimaculatus	*mitchellae*
walkeri	*sollicitans* [1]
	sticticus
Culex erraticus	*taeniorhynchus* [1]
nigripalpus	*thibaulti*
peccator	*tormentor*
pilosus	*triseriatus*
pipiens [2]	*trivittatus* [4]
quinquefasciatus	*vexans*
restuans	
salinarius	*Psorophora ciliata*
tarsalis [3]	*confinnis*
territans	*cyanescens*
	discolor
Culiseta inornata	*ferox*
melanura	*horrida*
	howardii
Mansonia perturbans	*varipes*
Orthopodomyia alba [5]	*Uranotaenia lowii*
signifera	*sapphirina*
Toxorhynchites rutilus rutilus [6]	
rutilus septentrionalis	

[1] Salt marsh species does not occur in southwestern Georgia.
[2] Reported only from North Georgia.
[3] Reported from Macon in 1943, not found in southwestern Georgia.
[4] Reported from Lee County in 1924, not found since that time.
[5] Reported from Georgia in 1945, not found in southwestern Georgia.
[6] Reported from Georgia before 1920, not found in southwestern Georgia since 1940.

ciliata hatched when *Aedes* larvae became abundant following early spring rains. After the areas became dry and were subsequently filled, large numbers of *Psorophora,* as well as *Aedes,* were produced.

ANOPHELES. Six species of *Anopheles* were found in southwestern Georgia. *A. quadrimaculatus* occurred in "permanent" limesink ponds throughout the area. This species may breed throughout the winter depending on severity of the cold (Bellamy, 1950). In this area, true hibernation was not observed; males died off and females became inactive during the winter for periods of several days. During the warm intervals, however, the females became active and fed (Zukel, 1949a, 1949b). In some instances ova were produced following winter feedings but frequently only fat bodies were produced. During a mild winter such as 1948–49 breeding apparently was not interrupted; fourth instar *A. quadrimaculatus* larvae and adult males and females were collected each month during the period. In more severe winters neither larvae nor adult males were collected from November until March.

During the winter of 1946–47, over 200 wild *A. quadrimaculatus* females collected from artificial resting stations were isolated individually and held in an area of the insectary which was heated during the day to approximately 70° F. and unheated at night. Each specimen was given an opportunity to feed daily from

TABLE 5.—Summary of information on occurrence of culicidae in southwestern Georgia

Genus and Species	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Anopheles barberi	-	-	-	-	-	-	-	-	IR	-	-	-
crucians	ITR	ITRBS	ITRS	ITBRS	ITBRS	ITRBS	ITBRS	ITRS	ITRS	ITRS	ITRS	ITBRS
georgianus	-	-	-	-	I	-	-	-	-	-	-	-
punctipennis	ITRS	ITRB	ITRS	ITRS	ITRBS	ITRS	ITR	ITR	ITRS	ITBRS	ITRS	ITR
quadrimaculatus	ITBRS	ITR	ITRS	ITRS	ITBRS	ITRBS	ITRS	ITRS	ITBRS	ITBRS	ITRS	ITRS
walkeri	-	-	-	-	-	-	T	T	T	-	-	-
Aedes atlanticus	-	I	-	TBIS	TBS	TBS	ITBRS	ITBS	TBS	TBS	TBS	-
canadensis	IT	IT	IT	ITB	TB	T	-	T	T	TS	-	S
cinereus	-	-	-	B	B	T	-	-	-	-	-	-
dupreei	-	-	-	TS	TS	T	TS	ITS	TS	TS	-	-
fulvus-pallens	-	-	-	-	TBS	TS	TS	TS	TS	TS	S	-
infirmatus	BR	-	ITBS	TBRIS	TBS	TBS	TBRS	TBRS	TBS	TBS	TBS	BS
mitchellae	ITBR	ITBRS	TIBS	ITBS	ITS	TS	TS	ITS	ITS	TS	TS	TBIS
sticticus	-	-	T	TBR	TBS	TBS	-	-	-	-	-	-
thibaulti	-	-	R	BR	RB	TS	TS	S	-	-	-	-
tormentor	-	-	-	-	TB	T	TI	T	T	T	-	-
triseriatus	I	I	ITR	ITBS	ITBS	ITBS	ITBS	ITS	ITS	IST	IS	I
vexans	ITBRS	ITRS	ITRS	ITBRS	ITBRS	ITBRS	TRBS	ITBRS	ITBS	TBRS	TBIS	ITS
Culex nigripalpus	-	-	I	S	TS	-	-	ITS	ITS	TS	T	IS
quinquefasciatus	I	-	IT	I	ITR	IRST	ITRS	ITRS	TIS	TRIS	ITRS	I
restuans	ITBRS	ITRS	ITRS	ITRS	ITS	TRIS	TRS	TS	ITS	ITRS	ITRS	ITRS
salinarius	-	-	-	-	-	-	-	-	-	-	T	-
territans	ITRS	ITRS	ITRS	ITRS	ITRS	ITRS	TS	ITS	TRIS	ITRS	ITS	ITS
(Melanoconion) sp.	TRS	TR	TRIS	TIRS	TRIS	TIRS	ITRS	TRIS	TRIS	TRIS	TRIS	RTS
erraticus	TR	R	I	IR	RIT	IR	ITR	ITR	ITR	ITR	IT	T
peccator	-	-	-	-	I	-	I	I	IT	TR	-	-
pilosus	-	-	-	-	-	-	TI	TI	IT	IT	T	-
Culiseta inornata	TIS	ITS	TI	TRS	TS	T	T	T	T	TS	TS	TSI
melanura	TR	TRS	ITRS	TRSI	TRS	TRS	TSR	TS	TS	TS	TS	TS
Mansonia perturbans	-	-	-	TBR	TBRS	ITBRS	TBRS	TRSB	TBS	TBRS	-	-
Orthopodomyia signifera	I	I	IR	ITS	IS	ITS	IS	ITS	ITS	IS	ITS	IT
Psorophora ciliata	-	I	IB	ITBS	TIBS	ITBS	ITBR	IBTS	ITSB	TISB	IT	-
confinnis	IBR	I	I	ITS	TIS	TBI	ITBS	ITBS	ITBS	ITBS	I	IR
cyanescens	-	-	-	ITB	BT	BTS	TI	IBTS	IB	BTS	-	-
discolor	-	-	-	IT	IT	TS	ITS	ITS	IT	T	-	-
ferox	-	I	TI	ITBS	TBIRS	TBRS	ITBS	ITBS	ITBS	TBRS	BST	-
horrida	-	-	-	-	B	TB	T	I	-	-	-	-
howardii	-	-	-	I	-	S	S	IBT	TB	TB	-	-
varipes	-	-	-	T	B	BT	BS	T	-	B	-	-
Toxorhynchites rutilus septentrionalis	I	I	I	I	I	I	IS	I	I	I	I	I
Uranotaenia sapphirina	TR	TR	ITR	ITR	ITRS	ITS	ITS	ITS	ITS	ITS	ITRS	TRS
lowii	-	-	-	-	-	-	-	T	T	T	T	-

T - Adults collected in light traps
R - Adults collected in resting stations
I - Immature stages collected
B - Adults collected biting
S - Adults collected in mechanical sweep nets

the hand or arm of the insectary technician; usually 3 to 5 specimens engorged fully. No ova were obtained, however, from the middle of December through February, although some specimens survived for more than 60 days and fed as many as 5 times. In a similar study conducted during January, February, and March, 1953 more than 100 wild female *A. quadrimaculatus* were collected from the same stations as in 1947 and placed in an open section of the insectary under essentially ambient conditions. In every instance the surviving females fed, developed ova, and oviposited. Low temperatures to which the specimens were exposed were about the same in 1947 and 1953. The high temperatures were different, however, since in 1947 the insectary was heated during the day. The higher daytime temperatures in 1947 may have stimulated more activity and feeding. But the greater temperature variations from day to night in this series may have accounted for the production of fat rather than ova. The mortality rate was low in the 1947 series but exceeded 50 percent in the 1953 series. Mortality in the 1953 series usually occurred among unfed specimens.

In January and February 1953 a series of female *A. quadrimaculatus* from the laboratory colony were fed in the insectary at 78° F. and held at a constant temperature of 50° F. All of these specimens developed ova.

In the field, populations of *A. quadrimaculatus* adults usually began to increase in April and rose sharply during May and June. Reductions usually occurred in July and August as a result of high summer temperatures detrimental to larval development (Goodwin and Love, 1957; Love and Whelchel, 1957) and rose again in September when temperatures moderated. Populations declined sharply when the minimum temperature fell below approximately 40° F.

Variations in the time when maximum density of *A. quadrimaculatus* occurred during comparable years of normal precipitation were related to early spring temperatures. Populations generally were higher in June or early July. The maximum height which the population reached was influenced by the length of time during which favorable developmental temperatures occurred. Moderate temperatures, averaging above 60° F. during February and March, permitted larval development and resulted in higher population levels during June than were noted when winter and early spring temperatures were lower. This effect of early spring temperature on summer populations has been observed also in the Tennessee Valley (Hess and Crowell, 1949).

A. crucians apparently overwintered principally as larvae, as observed in South Carolina (Frohne and Hart, 1949). In southwestern Georgia, however, some specimens complete development during warm periods of the winter, so that adult males and females were collected consistently throughout the winter from artificial resting stations adjacent to the breeding areas. Larvae of both *A. crucians* and *A. quadrimaculatus* developed at temperatures above 50° F.; lower temperatures were apparently lethal to most *A. quadrimaculatus* but not to *A. crucians*. Consequently, *A. crucians* adult populations increased immediately after temperatures moderated in the spring. *A. quadrimacu-*

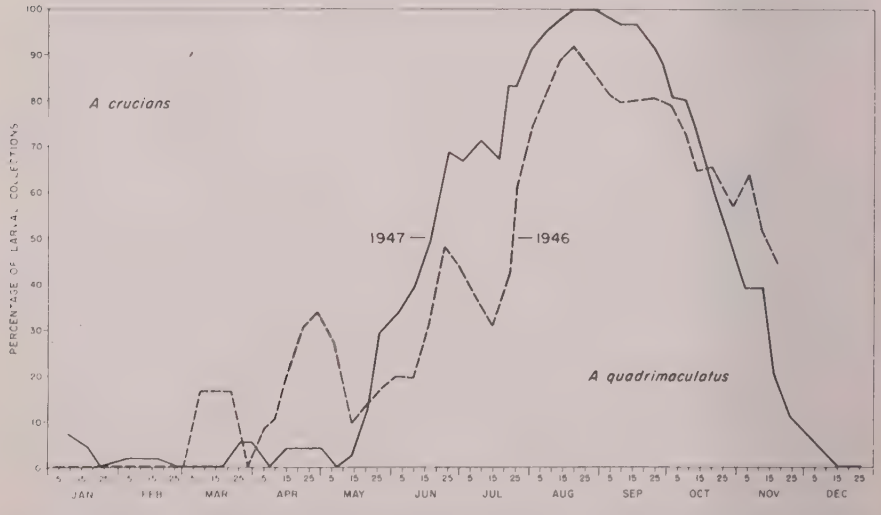

FIG. 8.—Relative abundance of *Anopheles quadrimaculatus* and *A. crucians* larvae in a permanent breeding area during 1946 and 1947. Area below the line shows percent of *A. quadrimaculatus*. Area above line shows percent *A. crucians*.

latus adult populations increased only after temperatures moderated for a period sufficiently long to permit development from egg to adult.

The relative abundance of *A. quadrimaculatus* and *A. crucians,* based on weekly collections of larvae in 1946 and 1947, from an area suitable for heavy production of both species is shown in Figure 8. Temperatures during these years were close to the average. *A. quadrimaculatus* was the predominant species from June through October, but few specimens were collected during the remaining months when *A. crucians* was more abundant. However, this pattern varied considerably in years with abnormal temperatures. During the winters of 1948–49 and 1949–50 monthly mean temperatures ranged from 3 to 12 degrees above the average shown in Table 2. *A. quadrimaculatus* larvae were collected regularly throughout both of these winters. The relative abundance of *A. quadrimaculatus* and *A. crucians* larvae in relation to mean water temperature during 1949 is shown in Figure 9. Above 75° F. the proportion of *A. quadrimaculatus* rose rapidly. Below 75° F. larval collections were always predominantly *A. crucians.*

A. punctipennis larvae and adults were most abundant during the winter months, but could be found in considerable numbers in suitable breeding areas throughout the year. In southwestern Georgia, *A. punctipennis* s.l. was represented by both the typical form and the "single haired" form, *A. p. perplexens* Ludlow. Bellamy (1956), after an exhaustive study, concluded that each form represents a distinct species. Ova of the two forms are easily recognized by the size and shape of the dorsal exposure of endochorion. In *A. punctipennis* ova this exposure is slipper-shaped and smooth-edged while in *perplexens* ova a much narrower area is exposed and the sides of the exposure are wavy. On fourth instar *perplexens* larvae, hair 2 on abdominal segments IV and V is predominantly single but is generally branched on *punctipennis.* Adults of *punctipennis* have longer wings than *perplexens* and the light subcostal spot is reduced in *perplexens.* Ecologically, *A. punctipennis* was found in a wide variety of temporary and permanent waters, but *A. perplexens* was apparently restricted to limestone springs and the streams formed by them.

Since no effort was made to separate the two forms before 1949, only *A. punctipennis* s.l. is considered in this report. Adult populations were low from June to September (Figure 10), rose sharply to a peak in October, receded in December when low midwinter water temperatures occurred, increased in February and reached the highest peak in March or April. The trend of larval abundance was the same as that of adults.

In the laboratory, ovarian development of *A. punctipennis* proceeded at reduced temperatures, but oviposition did not occur until the temperature was raised. Engorged adult females held at a constant temperature of 56° F. produced no ova within 15 days. However, specimens which were removed after 6 to 13 days' exposure to 56° F. temperature and placed at a constant temperature of 75° F. always oviposited within 2 to 16 hours.

The remaining three species of *Anopheles* in the area were collected infrequently. *A. barberi* was found occasionally in tree holes, the characteristic habitat of this species, but successive collections of *A. barberi* larvae could rarely be made from the same tree hole. *Anopheles georgianus* King was identified only once in larval material collected in the study area. Adults of this species are indistinguishable from *A. crucians.* During the winter of 1937–38 Bellamy (1939) collected *A. georgianus* larvae from Brooks, Sumter, Terrell, and Thomas Counties, all within 50–75 miles of the study area. Additional collections made subsequently in these counties indicate that larvae may be collected almost any time during the year. The classic habitat of this species is pasture-land seepage areas which are headwaters of small streams. In these habitats *A. georgianus* may be associated with a few *A. crucians*

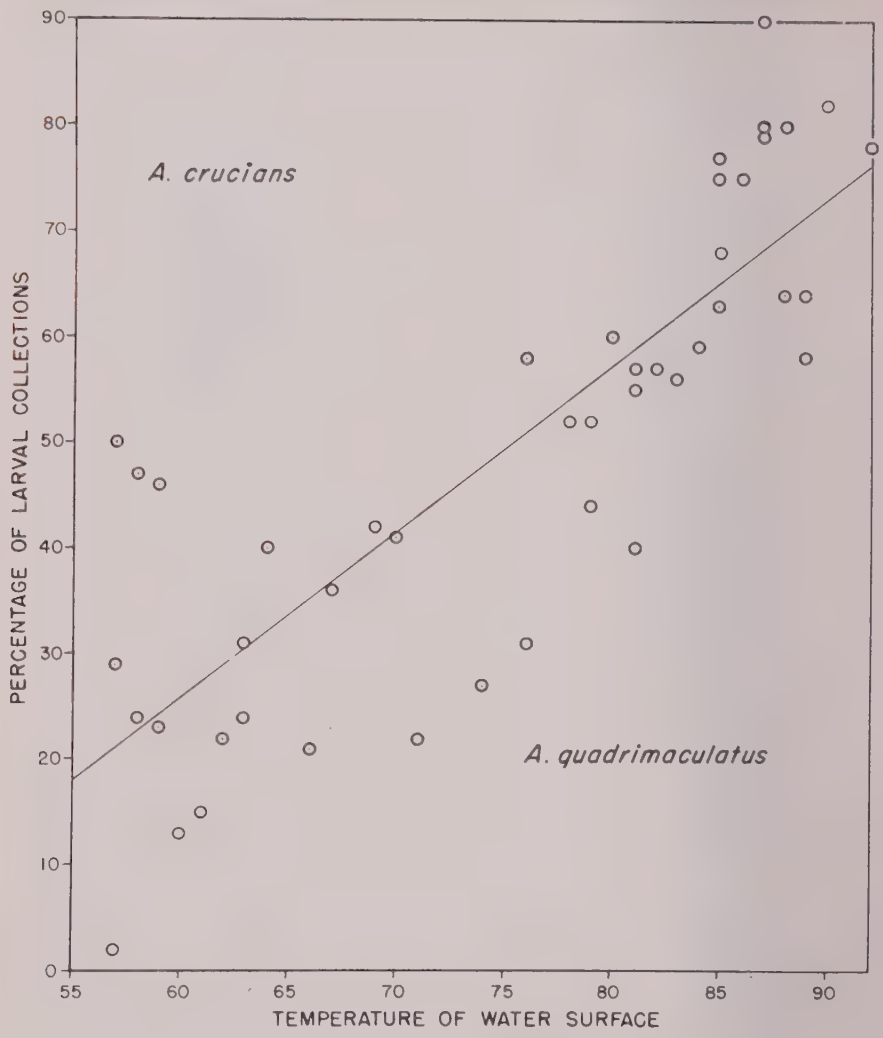

FIG. 9.—Relative abundance of *A. quadrimaculatus* and *A. crucians* larvae in a permanent breeding area during 1949 in relation to surface water temperature. Shown are individual collections (circled points) and line of least squares. Area below the line (or point) shows percent of *A. quadrimaculatus*. Area above line shows percent of *A. crucians*.

but seldom with any other species of *Anopheles*. *Anopheles walkeri* Theob. was collected with light traps 5 times in 18 years; larvae were not collected.

Ova of both *A. crucians* and *A. punctipennis* were observed to withstand desiccation. A soil sample removed from a pond basin which had been dry for the 10 previous days was held at room temperature and approximately 50 percent relative humidity in the laboratory for 24 hours to allow further drying. Fifty

Fig. 10.—Average season abundance of *A. punctipennis*, Baker County, Georgia

anopheline larvae, both *crucians* and *punctipennis*, hatched when the soil was flooded. Subsequent insectary studies showed that, as long as mud was sufficiently moist to maintain a film of water on the surface, *A. quadrimaculatus* ova would hatch in the usual time. Under the same conditions larvae would develop to the pupal stage although the rate of mortality was high (90–95 percent). No adults were obtained.

During 1947 and 1948 precipitin tests were made on ingested blood in freshly engorged *Anopheles*. Of 4,971 *A. quadrimaculatus* and 4,244 *A. crucians* tested, 4,616 *A. quadrimaculatus* and 3,897 *A. crucians* reacted to human, equine, porcine, bovine, or avian antisera. Results indicated that approximately 1 percent of each species had fed on humans, slightly more than 1 percent on birds, and nearly 98 percent on horses, cows, or pigs. Most specimens were collected in barns and sheds; few were found in or under houses.

CULEX. In southwestern Georgia, the genus *Culex* is represented principally by *C. territans* and *C. restuans* in the winter and by *C. p. quinquefasciatus, C. nigripalpus, C. erraticus, C. peccator*, and *C. pilosus*, during the summer.

C. territans and *C. restuans* are predominantly northern species, and south Georgia is near the southern limit of distribution. These species were seldom collected except during the late fall and early spring probably because of unfavorable high temperature during the remainder of the year. The seasonal occurrence of each species paralleled the distribution of *A. punctipennis* (Figure 10). Adults were most abundant from March to May and were collected rarely during the summer. The number of adults and larvae increased in August or September and the adults usually reached a secondary peak during November. The annual pattern of occurrence was consistent but density varied from year to year. During mild winters, e.g. 1948–49 and 1949–50, larval development apparently was not interrupted and adult populations were sustained at high levels from November through April. During more severe winters, the lower temperatures inhibit development but do not cause serious mortality among larvae. Adults are less active, however, less oviposition occurs,

larval populations are smaller, and adults emerge during a comparatively shorter period than during mild winters.

Larvae of both species were common throughout the winter months, *C. territans* in the ponds and *C. restuans* in artificial containers and small temporary rain pools. During dry periods *C. territans* also was found in artificial containers. Apparently, this species was highly selective in choice of oviposition sites. Egg rafts were obtained from pools 10 to 25 feet in diameter which contained decaying leaves but no eggs were present at the same time in similar adjacent pools which contained no debris.

The developmental time of *C. restuans* larvae varied with temperature; less mortality occurred at low than at high temperatures. At a constant temperature of 78° F. or higher, less than 40 percent of the *C. restuans* developed to the pupal stage. Those which survived completed the cycle from ova to adult in 10 to 15 days. When larvae were alternated at 24-hour intervals between water baths at 85° F. and 55 to 60° F., the developmental period was lengthened to 20 to 25 days and mortality was reduced to less than 20 percent. Larvae reared at a constant temperature of 55° F. to 60° F. required more than 30 days for development to the pupal stage, but mortality was only 14 percent.

Results of the laboratory studies were consistent with field observations. The average mean air temperatures during the periods when adult *C. restuans* were most abundant were 68.3° F. and 58.3° F. in October and November and 61.0° F. and 67.5° F. during March and April (Table 2, Figure 10). The higher spring peak was attributed to the longer period of temperatures favorable for larval development than preceded the fall peak.

The periods of maximum adult activity and oviposition, as indicated by light trap collections and daily collections of ova from selected breeding sites, occurred when the temperature was between 50° F. and 60° F. Activity declined steadily with lowering temperatures, and, at approximately 40° F., ova and adults were not collected.

In the summer, *C. restuans* in artificial containers was succeeded by *C. p. quinquefasciatus*. This species became very abundant around houses and barns but was found rarely in woodland areas. *C. p. quinquefasciatus* adults were taken in traps from March through November, and larvae were collected regularly from March through October; one collection of larvae was made in January 1950. A few hibernating adults were collected during the winter months by fumigating hollow trees.

Chickens apparently were the principal source of blood. This impression was corroborated by a laboratory study conducted during the summer of 1954. A large colony, reared from specimens collected in nature, was established in a cage 10 by 10 by 15 feet. Rabbits and birds were placed in the cage as constant sources of blood. Human blood was offered for one-hour intervals two or three times each day between 8 a.m. and 8 p.m. The locations of the rabbits and birds within the cage were changed each 24 hours so that the animals were placed at all possible elevations in relation to one another. Each morning a sample of freshly engorged females was collected and prepared for precipitin tests. None of nearly 200 specimens tested fed on either human or rabbit blood.

Culex nigripalpus was usually rare. With the exception of a single collection of larvae in March, specimens were observed only from August through November. Larvae occurred most frequently in ditches and small open pools but occasionally during the late summer months, large numbers were obtained from permanent ponds which had receded to depths of one to two feet. At this stage lush growths of floating and emergent vegetation were present. In these areas, more than one egg raft per square foot of water surface was sometimes oviposited in a single night. During periods of such heavy production, which lasted from 6 weeks to 2 months, *C. nigripalpus* adults

accounted for more than 50 percent of all mosquitoes in light trap collections.

Culex salinarius was collected in light traps in November 1942. A single male was identified early in the month and one female was found in a collection made approximately two weeks later. The two collecting stations were 15 miles apart. These two specimens represent the only record of this species from the study area.

The difficulty in specific identification of the *C. (Melanoconion)* group poses a problem to mosquito ecologists in the area. Until recently three species in south Georgia, *C. erraticus, C. peccator,* and *C. pilosus,* were considered members of this group. Each species apparently occupies a separate ecological niche but the three are indistinguishable as adults except by study of the male genitalia. After an intensive study of *C. (Melanoconion)* larvae, Foote (1954) revised the subgenus *Mochlostyrax* for some species including *C. pilosus.*

C. erraticus larvae succeeded *C. territans* in permanent ponds during the summer months. *C. erraticus* was the most common *Culex* in ponds from May to October; larvae were not collected in December, January, or February. *C. peccator* larvae were found most frequently in semipermanent, sunlit ponds which contained heavy growths of emergent vegetation. *C. pilosus* was collected only from roadside ditches and small temporary pools. Observations corroborated reports of others (King, *et al.,* 1939) that ova of *C. pilosus* withstand desiccation. Larvae were collected within a few hours after flooding from areas which previously were dry. Positive identifications of *C. pilosus* and *C. peccator* adults were made from light trap material collected from July to November. *C. erraticus* adults were identified from material obtained during each month of the year. Both *C. erraticus* and *C. peccator* were collected by fumigating hollow trees during the winter months. Because of the time involved, many collections were identified only as *C. (Melanoconion).*

CULISETA. Two species of *Culiseta* occurred in the area, *inornata* (Will.) and *melanura* (Coq.). Both were most abundant during the winter and early spring; *inornata* was readily attracted to light traps, but *melanura* was taken only occasionally (Love and Smith, 1957). However, in collections with mechanical sweep nets which utilized no attractant, the species were equally abundant. Both species were collected in greatest numbers with light traps during April, but specimens were collected occasionally throughout the year.

Female *Culiseta inornata,* collected in the field, oviposited readily in the insectary. Adults were held in 18- by 18- by 24-inch screen cages, and clear glass finger bowls half filled with tap water were placed on the bottom of the cages to receive the eggs. After hatching in 2 to 4 days at 78° F., larvae were placed in an aquarium. Larval development was completed in approximately 15 days, and adults emerged 6 to 10 days later. Mortality was low among larvae and pupae reared in the aquarium but was high when enamel pans were employed as for rearing *A. quadrimaculatus.* Mating was observed in many instances, but few females fed when offered rabbit, pigeon, human, and fox blood from cotton pledgets. No eggs hatched from several egg rafts obtained in the insectary.

Culiseta inornata larvae darted to the bottom of rearing pans or aquariums at the slightest disturbance and remained motionless for several minutes. This behavior probably accounted for the infrequency with which larvae of this species were obtained in routine larval collections.

Larvae of *Culiseta melanura* were collected infrequently from small grassy marshes or shallow permanent pools. Although light traps are only slightly attractive for this species, adults were taken in this manner each month during the year but were most abundant from February through May. In some years adults were not collected with light traps from April through December.

Laboratory studies substantiated the observations of Chamberlain *et al.* (1955)

that larvae collected from a cool environment require a short period of conditioning to enable survival at higher temperatures in the insectary. Larvae collected in November or December required as long as two months to complete development from third or fourth instar to adult. Development time was not affected by variations in length of the photoperiod. Females fed on a pigeon, but mating apparently did not occur in a cage 18 by 18 by 24 inches. The egg rafts deposited were distorted and failed to hatch. Reared adults survived as long as 3½ months under insectary conditions.

URANOTAENIA. U. sapphirina was generally the most common species of mosquito in permanent ponds from spring until fall. Larvae were found in almost any situation suitable for *Anopheles* and also in open sunlit ponds. Probably because larvae of *Uranotaenia* can survive and develop at higher temperatures than can *Anopheles,* adult populations did not drop during the hotter months as did anopheline populations. Larvae were usually found from March to November but were never collected in December, January, or February. Collections with light traps, which are strongly attractive to *Uranotaenia,* indicated that adults are first active in March and increased rapidly to a peak in July, when as many as 5,000 specimens were taken by a single light trap in one night. Populations receded slowly through September and dropped rapidly during October and November. Few adults were collected in light traps throughout the winter but many hibernating *U. sapphirina* females were collected by fumigating hollow trees. In one instance over 400 specimens were recovered from one tree. Largest numbers of specimens were found in trees having large hollow areas with small entrance openings (Zukel, 1949a). Few *Uranotaenia lowii* Theob. were identified.

MANSONIA. M. perturbans is the only species of this genus found in southwestern Georgia. Most of the permanent ponds provide suitable habitats for larvae of this species. Plants commonly associated with *Mansonia* were *Sururus cernuus* and *Pontederia cordata.* Adults of the single annual generation first emerge in April and almost immediately become abundant around breeding areas. Adults were prominent in light trap collections through June and were collected occasionally through October. Biting records indicate that adults are most active at dusk before light traps can be utilized effectively. *M. perturbans* fed readily on humans in shaded situations adjacent to breeding areas at any time of the day. Adults were never collected from October until April, which was probably the minimum time of larval development in this area. In the insectary at 78° F. ova were obtained from wild-caught females 6 to 8 days after feeding and in every instance ova required 5 days for hatching.

AEDES. Thirteen species of *Aedes* were found in southwestern Georgia. Their distribution and abundance was apparently governed by temperature and rainfall.

Ae. mitchellae and *Ae. canadensis* occurred primarily during the cooler months, but *Ae. mitchellae* was collected occasionally in light traps throughout the year. *Ae. vexans* was found from early spring until late fall. The remaining species of *Aedes* occurred primarily from April through August, but during mild winters breeding sometimes continued without interruption.

Deficient precipitation during the water-storage period (January, February, and March) from 1949 to 1954 depleted breeding areas. During one or more years of this period almost all ponds became dry. Suitable conditions for breeding of *Aedes* and *Psorophora* developed in most ponds when water levels were reduced. Consequently, populations of these mosquitoes were unusually large during the summers of 1950 to 1954 compared with populations from 1945 to 1950 when pond levels were high. During the years of normal precipitation *Aedes* and *Psorophora* spp. comprised less than 10 percent of light trap collections but during the dry years these species accounted for more than 90 percent of the speci-

mens collected. Also, during the drier years, total mosquito catches in light traps were 80 percent higher than in the years when average breeding conditions existed (Smith and Love, 1956).

The seasonal distribution and abundance of *Ae. mitchellae* and *Ae. canadensis* was similar to *A. punctipennis* (figure 10). Larvae of these species were found in temporary grassy pools. Ova were deposited in areas subject to flooding and larvae hatched when water accumulated after winter or early spring rains. Hatching was sometimes delayed and development of larvae prolonged by severe cold. As observed for the winter *Culex* species, larvae sometimes accumulated during the winter and caused an explosive increase in adult populations early in the spring. *Ae. vexans* sometimes occurred early in the spring, but became most abundant in April or later. At the peak of production some rain-filled depressions literally teemed with larvae. *Ae. atlanticus* and *Ae. infirmatus* sometimes became locally abundant from May through September when woodland pools were filled. *Ae. sticticus* (Meig.), *Ae. tormentor* D. and K., *Ae. thibaulti* D. and K., *Ae. fulvus pallens* (Ross), *Ae. dupreei* (Coq.), and *Ae. cinereus* Meig. occurred in lesser numbers but became locally abundant for short periods between May and July. *Ae. triseriatus* overwintered as larvae observed in tree holes at most times of the year. Larvae were also found in artificial containers in shaded situations. Adult *Ae. triseriatus* were collected infrequently by light traps or by biting collections from March through summer. Most other *Aedes* were readily attracted to light traps.

Ae. aegypti (L.) occurred in artificial containers only in the urban areas. A survey of one city in October 1956 revealed larvae in 88 percent of the city blocks and at over 40 percent of the individual residential or business premises examined. Studies in this area indicated that *Ae. aegypti* larvae probably survived the winter in outdoor containers (Smith and Love, 1958).

Insectary studies showed that drying and reflooding were not necessary for hatching of all *Aedes* ova. *Ae. vexans* ova, which were deposited directly on water and remained for 10 days without hatching, usually hatched readily after transfer to a hatching medium consisting of an infusion of dog biscuits and water which had aged for two or three days to reduce oxygen concentration. Ova did not hatch until they were submerged in the medium. This occurred readily when a drop of water fell on the egg. Hatching followed within 15 minutes. Studies of hatching also showed that *Ae. vexans* ova in nature did not hatch when flooded during the winter when temperatures were low. However, ova remained viable for at least several months. In one instance, samples were removed in May from the bottom of a pond which had held water for four months following a winter rain. *Ae. vexans* larvae hatched after the samples were dried thoroughly and reflooded.

Studies were made on the effect of light on development of *Ae. triseriatus* following observations that larval stages were prolonged in a laboratory colony (Repass, 1953) during the fall (Love and Whelchel, 1955). It was found that shortening the photoperiod in the fall stimulated production of ova from which photosensitive larvae developed. These larvae pupated only when the duration of daylight was approximately 12 hours or more. Larvae from ova produced at other times of the year did not show such sensitivity and were capable of completing development in total darkness. In the insectary where the photoperiod approximated that in nature, the first pupae were obtained from overwintering larvae in late January. Pupation continued through February and 50 to 60 percent of the larvae pupated during March. Field collections made concurrently with the laboratory study showed that 70 to 80 percent of the *Ae. triseriatus* in natural tree holes were in the pupal stage when pupation in the laboratory was complete. Two weeks later no larvae or pupae were present in the same tree holes. As a re-

sult of these studies, continuous production of larvae was obtained by regulation of the photoperiod to which adults were exposed.

PSOROPHORA. In southwestern Georgia, the genus *Psorophora* is represented by 8 species (Table 3). Larvae developed in temporary wheel ruts and roadside ditches. Under favorable conditions many species developed from ova to adult in 5 to 7 days. *Aedes* sp. usually predominated in the intermittent ponds but *Psorophora* sometimes occurred in these situations.

Ciliata, confinnis, and *ferox* were the most common *Psorophora* with respect to frequency of collection, number of specimens, and period of occurrence. The average seasonal distribution was from May through October, but moderate temperatures in early spring or late fall extended this range from February or March through November. Although encountered less frequently, *P. cyanescens* and *P. discolor* sometimes became locally abundant following summer rains. *P. horrida, P. howardii,* and *P. varipes* were collected infrequently and in small numbers. Prior to 1955 *P. discolor* had been collected on only two occasions. During this year specimens were identified regularly, and, in 1956 *P. discolor* was as prominent as *P. confinnis* in breeding areas which had previously produced only *P confinnis.* In 1957 *P. discolor* was collected regularly but was not so abundant as during the two previous summers.

Females of all *Psorophora* spp. in the area were fierce biters and fed readily at any time of the day in shaded situations near breeding areas. Specimens rarely were observed biting other than in wooded areas during the day. Of the more common species of *Psorophora, confinnis* and *ciliata* were readily attracted to light traps but few *ferox* were collected in this manner.

ORTHOPODOMYIA and *TOXORHYNCHITES. O. signifera* and *T. r. septentrionalis* were found throughout the area in tree holes and shaded artificial containers. Adults of *O. signifera* were collected infrequently in light traps. Specimens rarely fed in the laboratory. A few individuals fed on an immobilized bird held overnight in the cage, but none fed on humans, rabbits, chickens, or caged birds or from cotton pledgets soaked with blood from these animals. *O. signifera* overwintered as photosensitive larvae and pupated only when days lengthened in the spring (Baker, 1935). Larvae were collected each month of the year.

T. r. septentrionalis adults were rarely observed in nature. Specimens were collected twice in light traps and on two occasions a single adult was observed on the wing in a densely wooded area. Larvae of the species can withstand temperatures below freezing. Specimens held at approximately 30° F. in a block of ice for a period of one week apparently suffered no ill effect. Larvae were killed within 24 hours, however, when exposed to temperatures of approximately 0° F. The species overwinters as larvae or ova. Some overwintering larvae of *T. r. septentrionalis* collected in the field remain in the fourth instar for several weeks after being placed in the insectary at 80° F. Other specimens, however, pupate within a few days.

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Charges were made for extra pages in this paper.

EFFECTIVENESS OF NEW INSECTICIDES AGAINST MOSQUITO LARVAE [1]

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Introduction. In California, the current mosquito control technology is predominantly directed toward the abatement of pest and vector species in their aquatic habitats. Proper management of the larval sources in limited areas has yielded good results. This end has usually been achieved by drainage and other effective engineering schemes. Utilization of biotic control agents such as mosquito fish and other predators under optimum and suitable environmental conditions is also aiding mosquito control in California. Although source reduction and biotic control measures are employed whenever possible, the use of chemical larvicides constitutes a major segment of current mosquito control technology in California.

The physical and biological components of the environment in which mosquito larvae are controlled with larvicides are complex. They vary from one location to another even in a limited area and do not remain constant in time. The types of breeding sources harboring mosquito larvae are diverse too, and no single remedy can be found or recommended for effective control under all circumstances. Insecticidal treatment of larval sources located in agricultural fields poses certain problems pertaining to the residues of the larvicides on food and forage crops. Many effective and choice larvicides cannot be used routinely on crops without obtaining information on the residue levels and the proper establishment of tolerances for the larvicides on the crops to be treated for mosquito control.

There is a need for the development of a variety of larvicides to be employed under diverse environmental conditions. The need for short-lived materials in agricultural areas is obvious. On the other hand, residual larvicides are preferred in situations where residues on crops do not pose a serious problem. Safety of larvicides to predators of mosquito larvae is another consideration which merits attention. Materials having low mammalian toxicity are also sought for and their use in urban larviciding programs is greatly desired.

The occurrence in mosquitoes of resistance to malathion and parathion further emphasizes the need for substitute materials. Materials having desirable attributes are very few and systematic search for such larvicides should pay off in the long run. To gather information on the behavior and activity of various new materials, studies were conducted on the biological activity of candidate materials against mosquito larvae. As a result of studies conducted in both laboratory and field during the 1959 season, several highly effective and promising mosquito larvicides were found (Mulla *et al.* 1960). The present studies are a continuation of the earlier studies and deal with the effectiveness of several other materials. The toxicity of all the larvicides evaluated in the field during 1959 (Mulla *et al.* 1960) and during the present studies was also determined against the mosquito fish *Gambusia affinis* (Girard and Baird) and will be published elsewhere (Mulla and Isaak 1961). Additional observations on the toxicity to mosquito larval predators of the larvicides which were evaluated in the field earlier (Mulla *et al.* 1960) were also recorded. Such observations, although not definitive in nature and scope, will materially aid in the selection of a proper larvicide.

[1] These studies are supported by grants-in-aid from Consolidated, Fresno, Kern, and Westside Mosquito Abatement Districts in California. Paper No. 1296, University of California Citrus Experiment Station, Riverside, California.

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The effectiveness of most of the insecticides was determined in the laboratory prior to extensive field evaluation. The laboratory information facilitated the determination of approximate dosages of the materials for field evaluation.

Materials and Methods. The materials were evaluated in the laboratory against 4th instar larvae of *Culex pipiens quinquefasciatus* Say in the same manner as described earlier (Mulla *et al.* 1960). Twenty-five 4th-instar larvae were placed in 100 ml. of tap water in 6-ounce paper cups. Acetone solutions of the insecticides were added to the cups and larval mortality was recorded 24 hours after exposure. The percent mortality was plotted on probit log scale and the points fitted visually with a straight line. Each treatment was replicated three times and each material was evaluated on two or three different occasions.

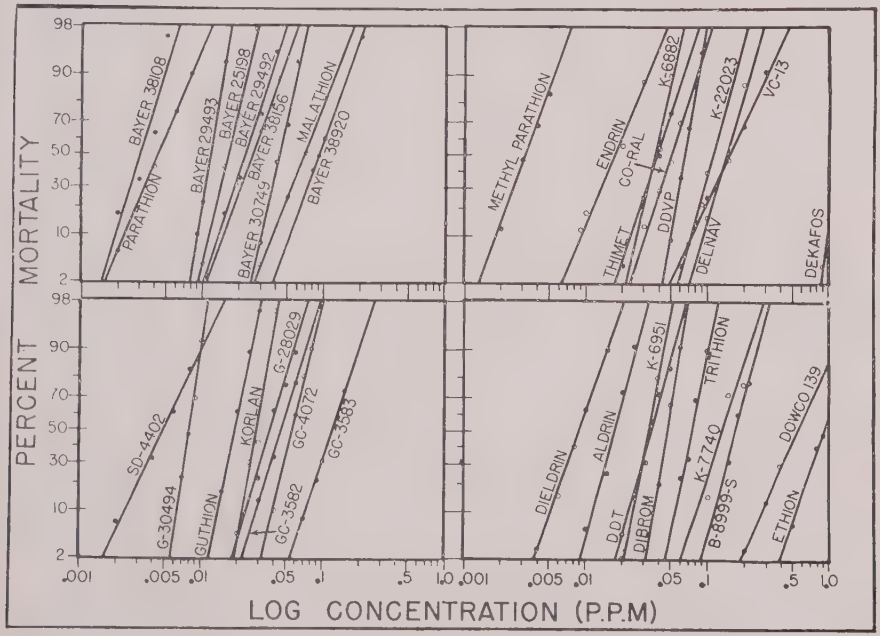

Fig. 1.—Dosage response lines of several insecticides evaluated in the laboratory against susceptible 4th-instar larvae of *Culex p. quinquefasciatus*. Parathion SD-4402, Bayer 29493, DDT, and malathion are included for comparison.

Figure 1 shows dosage response lines of 33 insecticides not previously reported on; for comparison, the dosage response lines of parathion, Bayer 29493[4] (Baytex), DDT, SD-4402 and malathion are also included.

Dosage response lines of a few compounds manifesting high biological activity against mosquito larvae are at the extreme left of the figure, near the activity level of parathion. In this category of highly promising materials are Bayer 38108, Bayer 29493, Bayer 25198, methyl parathion, G-30494, SD-4402 and dieldrin. Bayer 34042, also in this category, is not shown on the figure.

Three materials, Dowco 139 (Zectran), ethion, and Dekafos, with low biological activity against mosquito larvae have dosage response lines located to the extreme right. These materials have such low activity against larvae that their use can be justified only under special conditions.

Most of the remaining materials showed a moderate to high degree of activity. The dosage response lines of these materials are clustered along the dosage response lines established for DDT and malathion. Many promising and versatile materials belong to this group of larvicides.

The effectiveness of all those materials presented in figure 1 and of some of those evaluated in the laboratory during the earlier studies (Mulla *et al.* 1960) but not shown in figure 1 can be more fully visualized in terms of their LC50 and LC90 activity levels against mosquito larvae. This information for materials evaluated thus far is presented in Table 1.

As a general rule, materials manifesting biological activity lower than that of malathion against mosquito larvae do not hold much promise for general use in California larviciding programs. Higher dosages (0.5 lb. or more/acre) of these materials would usually be required, which would not fit into a program aimed at controlling mosquito larvae in agricultural areas; probably such high rates for some of the materials would result in undesirable residues on food and forage crops. Also, the cost of such high rates would preclude their use over wide areas. A few of the materials in this category, however, by virtue of their stability in water, may be applied at economically feasible rates that will yield desired results. These compounds with lower activity may also have a place in the control of resistant strains where other conventional materials do not yield adequate control.

The evaluation of several organophosphate insecticides in the laboratory showed that methyl analogues of a number of insecticides manifested slightly more activity than the ethyl analogues (Table 2). But field evaluation of the activity of methyl and ethyl analogues, although showing the same tendency, has not progressed for many of the analogues to establish this trend definitely. However, ethyl analogues are considered more stable in the field and, therefore, may be used in situations requiring long-lasting materials. Bayer 25141, Ethyl Guthion, and Trithion had considerable residual activity against *Hippelates collusor* in soil (Mulla *et al.* 1961). Possibly these materials will show the same trend when they are used in mosquito larviciding programs.

The results in Figure 1 and Table 1 are presented to provide background information. Laboratory evaluation of candidate larvicides yields useful information on the general trend of activities of insecticides against mosquito larvae. The present procedure of exposing the larvae for 24 hours to the toxicants has been chosen for convenience. Such a short exposure period, however, may fail to discover the potential toxic effects of slow-acting and stable materials. Such materials may even be overlooked in field evaluation where 24-hour mortality counts are taken. The addition of acetone solutions of water-insoluble toxicants to water for laboratory evaluation may result in precipitation or separation of the toxicants and thus reveal some toxicants to be in-

[4] Chemical designations of proprietary or experimental compounds, are reported before "References Cited."

TABLE 1.—Effectiveness of various insecticides against 4th instar larvae of *Culex pipiens quinquefasciatus* [1] in the laboratory

MATERIALS	LC_{50} (P.P.M.)	LC_{90} (P.P.M.)	MATERIALS	LC_{50} (P.P.M.)	LC_{90} (P.P.M.)
BAYER 38108	0.0031	0.0078	BAYER 30749	0.043	0.061
PARATHION	0.0045	0.0082	PHORATE (THIMET)	0.040	0.064
SD-4402	0.0050	0.010	BAYER 25141	0.046	0.064
G-30494	0.0082	0.010	DIBROM	0.048	0.064
BAYER 29493	0.0110	0.014	GC-3582	0.046	0.070
DIELDRIN	0.0088	0.015	CO-RAL	0.051	0.072
BAYER 34042	0.0115	0.018	METHYL TRITHION	0.050	0.081
BAYER 25198	0.016	0.022	GC-4072	0.058	0.081
ALDRIN	0.017	0.025	DDVP	0.066	0.084
GUTHION	0.019	0.026	TRITHION	0.072	0.10
E.N. 18133	0.023	0.031	MALATHION	0.068	0.12
ENDRIN	0.017	0.032	BAYER 38920	0.09	0.16
BAYER 29492	0.023	0.036	DOW K-22023	0.11	0.17
RONNEL (KORLAN)	0.029	0.038	GC-3583	0.12	0.21
ETHYL GUTHION	0.031	0.041	DOW K-7740	0.14	0.21
BAYER 38156	0.027	0.043	DELNAV	0.15	0.23
BAYER 22408	0.031	0.046	VC-13	0.15	0.30
DOW K-6951	0.034	0.046	B-8999-S (ASP-51)	0.18	0.24
AC-5727	0.036	0.052	DOWCO 139	0.58	1.2
DOW K-6882	0.038	0.052	ETHION	0.90	2.0
DDT	0.035	0.053	DEKAFOS	1.30	1.5

[1] A susceptible strain, colonized from a colony which was maintained at Kern Mosquito Abatement District, Bakersfield, California.

effective. Such materials when used in the field as emulsifiable concentrates may yield promising results. Settling of some toxicants in the testing cups, absorption on the waxed surface of the cups, and codistillation with water are some variables which may render the results doubtful.

FIELD EVALUATION. Many of the materials listed in table 1 have already been evaluated in the field. Results of field trials with some of the materials are not conclusive, but a general trend of the activity of the materials against mosquito larvae has been established. Phorate (Thimet), Guthion, Trithion, ronnel, Co-

TABLE 2.—The toxicity of methyl and ethyl analogues of some organophosphorus insecticides to 4th-instar larvae of *Culex p. quinquefasciatus* in the laboratory [a]

Methyl analogues			Ethyl analogues			Toxicity of methyl/ethyl	
Material	LC50 (p.p.m.)	LC90 (p.p.m.)	Material	LC50 (p.p.m.)	LC90 (p.p.m.)	LC50	LC90
Methyl parathion	0.0033	0.0053	Parathion	0.0045	0.0082	1.4	1.6
G-30494	0.0082	0.010	G-28029	0.038	0.060	4.6	6.0
Bayer 29493	0.011	0.014	Bayer 29492	0.023	0.036	2.1	2.6
Bayer 25198	0.016	0.022	Bayer 25141	0.046	0.064	2.9	2.9
Guthion	0.019	0.026	Ethyl Guthion	0.031	0.041	1.6	1.6
Dow K-6951	0.034	0.046	Dow K-6882	0.038	0.054	1.1	1.2
Methyl Trithion	0.050	0.081	Trithion	0.072	0.10	1.4	1.3

[a] Susceptible strain.

Ral, DDVP, and Delnav have been evaluated in the field against *Aedes nigromaculis* and *Culex tarsalis* (Isaak 1956, Gjullin and Lewallen 1958, Lewallen 1958); Guthion was highly effective against *A. nigromaculis* larvae. SD-4402, Bayer 29493, E.N. 18133, Bayer 24408, AC-5727, Bayer 25141, G-30494, and Methyl Trithion were evaluated against *C. tarsalis* and *A. nigromaculis* larvae (Lewallen and Gjullin 1960, Mulla *et al.* 1960). SD-4402, Bayer 29493, E.N. 18133, and Bayer 25141 were highly effective larvicides. A few materials were evaluated in olive vats against *C. p. quinquefasciatus and C. peus* (Mulla 1961). Results of trials with several other materials in the San Joaquin Valley against *C. tarsalis* larvae are presented in this paper. Field investigation of the remaining highly active materials is in progress.

In field evaluation emulsifiable concentrate formulations of the materials were used for preparing the dilute sprays. Size of plots was $\frac{1}{16}$ acre and the spray was applied by hand sprayers at the rate of $\frac{1}{2}$ gallon per plot or 8 gallons per acre. Small hand sprayers with TeeJet No. 8002 nozzles were used for applying the sprays; pressure was maintained at 20 to 30 pounds per square inch.

Larval mortality was assessed by dippings; 10 to 15 dips were taken before and 24 hours after treatment of the plots. Only 4th-instar larvae of *Culex tarsalis* were counted. Observations on the presence of other stages were also made and recorded in the tables. The percent control based on the 4th-instar larval mortality was arrived at by comparing the average posttreatment counts with the average pretreatment counts.

The plots were nonreplicated, but certain dosages of some materials were run two or three different times under varied conditions. In this manner the general trend of activity of the materials was established.

All the field trials were conducted in the southern part of San Joaquin Valley, California, in cooperation with Kern Mosquito Abatement District. Some of the trials were conducted at a duck club 10 miles northwest of Wasco. Here, salt grass and other plants covered from 20 to 50 percent of the water surface. The remaining tests were conducted in artificial ponds constructed and maintained by the Kern Mosquito Abatement District. The ponds are 15 miles south of Bakersfield, near Greenfield, in an intensively farmed area. Each pond is $\frac{1}{16}$ acre and the water surface is clear of vegetation. The water depth ranged from 6 to 8 inches; water pH was 7.5. Water temperature during the tests varied from 80° F. to 90° F. during the day.

DUCK CLUB TRIALS. In the duck club trials Bayer 25198 and Bayer 38156 were highly effective against mosquito larvae (Table 3).

Bayer 38156, although highly effective in the duck club trials, did not prove as effective when evaluated in the ponds

TABLE 3.—Field trials with new insecticides against 4th-instar larvae of *Culex tarsalis* (Donald Duck Duck Club, Wasco, California)

Insecticide and formulation	Dosage, lbs. tox./acre	Avg. no. larvae/dip		% Control
		Pre-treatment	Post-treatment	
Bayer 25198 50% EC[a]	0.01	11.5	4.9	58
	0.025	13.1	0.3	98
	0.050	9.3	0.0	100
Bayer 38156 50% EC[a]	0.01	4.3	0.8	81
	0.025	3.0	0.0	100
	0.050	2.3	0.0	100
GC-4072 50% EC	0.10[b]	10.0	3.6	64
	0.20[b]	8.3	0.0	100
	0.40[c]	11.3	0.0	100

[a] Insect predators such as mayfly naiads, adults and larvae of diving beetles and backswimmers were alive in all treatments of these materials.
[b] Backswimmers both dead and alive. Dytiscid adults alive. Water boatman, mayfly naiads, and mosquito adults dead on water surface. Few mosquito pupae also dead.
[c] All predators dead in plot treated at this rate; mosquito pupae also dead.

(see Table 5) or in olive vats; whereas Bayer 25198 did prove effective in the olive vats (Mulla 1961). Therefore, the status of Bayer 38156 in mosquito control is not yet clearcut. Further trials will be necessary to understand clearly the biological activity of this material against mosquito larvae in the field.

Compound GC-4072 also proved effective but not as effective as Bayer 25198 and Bayer 38156 (see Table 1). GC-4072 proved slightly more effective than its structural isomer GC-3583 in the laboratory (see Table 1); this trend held true in the field as well. Both materials seem to be residual in water and studies on their residual effectiveness are in progress. GC-4072 was harmful to aquatic insects that are assumed to be predators of mosquito larvae. Both materials were toxic to the mosquito fish at larviciding dosages (Mulla and Isaak 1961).

Dow K-6882, an organic amidophosphate, had appreciable activity even at the

TABLE 4.—Field trials with new Dow Chemical Company compounds against 4th-instar larvae of *Culex tarsalis* (Donald Duck Duck Club, Wasco, California)

Insecticide and formulation	Dosage, lbs. tox./acre	Avg. no. larvae/dip		% Control
		Pre-treatment	Post-treatment	
Dow K-6882 25% EC[a]	0.05	8.8	1.0	88
	0.075	11.3	0.1	99
	0.10	7.2	0.5	93
	0.20	16.5	0.0	100
Dowco 139 25% EC[b]	0.05[c]	4.0	4.5	0
	0.10[c]	3.3	3.1	7
	0.20[c]	2.3	1.1	52
	0.40	8.7	1.8	79

[a] Predacious diving beetles were alive in all plots treated with this material.
[b] Insect predators such as mayfly naiads, adult and larval diving beetles and backswimmers were alive in all treatments with this material.
[c] Large numbers of second- and third-instar larvae of *C. tarsalis* were alive in these treatments, indicating the ineffectiveness of this material at the indicated dosages.

lowest dosage used against the larvae (Table 4). It manifested considerable activity against *Culex* larvae breeding in olive vats also (Mulla 1961). It proved harmless to some aquatic predacious insects at the larviciding dosage. Dowco 139 proved much less effective especially at the three lowest dosages against the larvae than the former compound; higher dosages of this material were needed for complete kill of mosquito larvae in the ponds (see Table 5). At all dosages employed in the duck club tests Dowco 139 proved harmless to aquatic predacious insects.

Bayer 25198 proved more effective than its ethyl analogue (Bayer 25141) against 4th-instar larvae of *Culex p. quinquefasciatus* in the laboratory. This trend was also noticed in the field trials conducted last year (Mulla *et al.* 1960) and in the current studies. In field trials, Bayer 25198 was 4 times as toxic as Bayer 25141. The biological activity of Bayer 29493 (Baytex) and Bayer 25198 against mosquito larvae in the laboratory (see Table 1) and field was essentially the same. Both materials were investigated for their toxicity against the mosquito fish and were relatively safe (Mulla and Isaak 1961). These three Bayer compounds were relatively harmless to insect predators of mosquito larvae at effective larviciding dosages.

BREEDING PONDS TRIALS. The ponds were constructed to reduce variation caused by mixing of water and diffusion of toxicants from one plot to the other. Variation due to plant cover was also eliminated in the ponds. The ponds were located in an agricultural area where parathion applications for mosquito larval

TABLE 5.—Control of 4th-instar larvae of *Culex tarsalis* with insecticides applied to breeding ponds (Greenfield, California) [a]

Materials and formulation	Dosage, lbs. tox./acre	Avg. no. larvae/dip		% Control
		Pre-treatment	Post-treatment[b]	
Parathion 50% EC	0.0005	157.0	165.0	0
	0.001	122.0	20.0	84
	0.003	115.0	0.0	100
	0.005	27.6	0.0	100
Dekafos 25% EC	0.20	14.5	15.5[c]	0
	0.40	11.5	6.0[c]	48
	0.80	6.1	0.3[c]	95
	1.60	15.8	0.3	98
K-6882 25% EC	0.01	5.6	7.7[c]	0
	0.05	4.1	0.2	95
	0.10	16.1	0.2	99
Dowco 139 25% EC	0.10	16.1	7.7[c]	57
	0.20	13.6	3.0[c]	80
	0.40	12.1	2.1	83
	1.0	17.8	0.0	100
Bayer 38156 50% EC	0.008	2.4	0.7[c]	68
	0.01	10.5	3.0[c]	72
	0.02	25.1	1.6[c]	92
	0.05	6.9	0.5[c]	93

[a] Water depth in the ponds was 6 to 8 inches, and water pH was 7.5. Water temperature measured during the day ranged from 80° to 90° F.

[b] All insect predators such as damselfly larvae, dytiscid larvae and adults (diving beetles) and dragonfly naiads were alive in all the treatments. Dead mosquito pupae were not observed in the treatments.

[c] Young mosquito larvae (1st through 3rd stage) were also alive in these treatments, thus indicating the ineffectiveness of the materials at these dosages.

control have been made for 7 or 8 years.

Parathion, Dekafos, Dow K-6882, Dowco 139, and Bayer 38156 were evaluated in the ponds against 4th-instar larvae of *Culex tarsalis* (Table 5). Parathion proved highly effective beyond expectation. Many mosquito abatement agencies in California have applied and are applying parathion at the rate of 0.1 pound per acre as a mosquito larvicide. No published work indicates that this dosage is the minimum effective rate for mosquito control in California. The dosage yielding kill less than 100 percent had not been established prior to the wide use of parathion as a mosquito larvicide. In the current studies a dosage as low as 0.003 pound per acre of parathion resulted in complete kill of the larvae in the ponds. Whether this susceptibility of the population to parathion is due to a reversal of the tolerance level or was present at the beginning of the larvicidal applications is not known. The dosages of parathion were applied late in the season when water temperature ranged from 55° F. to 60° F. Tests conducted earlier in the season showed complete kill of the larvae at 0.005 to 0.0075 pound per acre of parathion. This information is rather significant in view of the somewhat increased tolerance of *Aedes nigromaculis* to parathion in certain parts of the San Joaquin Valley (Lewallen 1960). Further studies on mosquito population in the ponds as well as on populations in other areas are contemplated for the future.

Dekafos proved effective but only at high dosages. Rates as high as 1.6 pound per acre or higher would be needed for complete kill of the larvae. It showed no appreciable activity against the young instar larvae at 0.8 pound per acre or lower rates. Dow K-6882 manifested appreciable activity in the ponds against the larvae and showed the same trend of activity observed in the duck club and olive vat treatments. Dowco 139 proved effective but at the relatively high dosage of 1.0 pound per acre. At the lowest two dosages this material did not show much activity against the young instar larvae.

Bayer 38156 did not prove as effective in the ponds as it did in the duck club studies. Early instar larvae were alive at all treatment levels of this material. Whether this difference in activity is due to mosquito population characteristics or to environment will be further investigated.

Acknowledgments

The able assistance and valuable suggestions of Arthur F. Geib, manager of the Kern Mosquito Abatement District, Bakersfield, California, during the course of these studies are very much appreciated. The authors also acknowledge the assistance of other personnel of that dis-

Chemical Designations of Proprietary Materials

AC-5727	*m*-isopropylphenyl *N*-methyl carbamate
B-8999-S (NPD)	Tetra-*n*-propyl dithionopyrophosphate
Bayer 22408	naphthaloximido-*O,O*-diethylphsophorothioate
Bayer 25141	*O,O*-diethyl *O*-*p*(methylsulfinyl)phenyl phosphorothioate
Bayer 25198	*O,O*-dimethyl *O*-*p*-(methylsulfinyl)phenyl phosphorothioate
Bayer 29492	*O,O*-diethyl *O*-[4(methylthio)-*m*-tolyl)] phosphorothioate
Bayer 29493	*O,O*-dimethyl *O*-[4 (methylthio)-*m*-tolyl)] phosphorothioate
Bayer 30749	*O*-ethyl *O*-*p*-ethylsulfinylphenyl methylphosphonothioate
Bayer 34042	*O*-ethyl *O*-(3-methyl-4-methylthiophenyl) *N*-methyl phosphoramidothionate
Co-Ral ®	*O,O*-diethyl *O*-(3-chloro-4-methyl-7-coumarinyl) phosphorothioate
DDVP	dimethyl 2,2-dichlorovinyl phosphate
Dekafos ®	3-pentadecylphenol *O,O*-diethylthiono phosphate and related compounds

Delnav ®	2,3-*p*-dioxanedithol *S,S*-bis (*O,O*-dimethyl phosphorodithioate)
Dibrom ®	1,2-dibromo-2,2-dichloroethyl dimethyl phosphate
Dowco 139 (Zectran ®)	4-dimethylamino-3,5-xylol-*N*-methylcarbamate
Dow K-6882	*O*-ethyl *O*-(2,4,5-trichlorophenyl) methyl phosphoramidothioate
Dow K-6951	*O*-methyl *O*-(2,4,5-trichlorophenyl) methyl phosphoramidothioate
Dow K-7740	*O*-methyl *O*-(2,4,5-trichlorophenyl)phosphoramiothioate
Dow K-22023	*O*-methyl *O*-(2,4-dichlorophenyl) isopropylphosphoramidothioate
EN 18133	*O,O*-diethyl *O*-(2-pyrazinyl) phosphorothioate
Ethyl Guthion ®	*O,O*-diethyl *S*-4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl phosphorodithioate
G-30494	*O,O*-dimethyl-*S*-2,5-dichlorophenyl mercaptomethyl phosphorodithioate
GC-3582	Diethyl-1-(2,5-dichlorophenyl)-2,2-dichlorovinyl phosphate
GC-3583	Diethyl-1-(2,5-dichlorophenyl)-2-chlorovinyl phosphate
GC-4072	Diethyl-1-(2,4-dichlorophenyl)-2-chlorovinyl phosphate
Guthion ®	*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl phosphorodithioate
Korlan ® (ronnel)	*O,O*-dimethyl *O*-2,4,5 trichlorophenyl phosphorothioate
Methyl Trithion ®	*O,O*-dimethyl *S*-(*p*-chlorophenylthiomethyl) phosphorodithioate
Thimet ® (phorate)	*O,O*-diethyl (*s*-ethyl mercaptomethyl) dithiophosphate
Trithion ®	*O,O*-diethyl *S*(*p*-chlorophenylthiomethyl) phosphorodithioate
SD-4402	1,3,4,5,6,7,8,8-octachloro 3a,4,7,7a-tetrahydro-4,7-methanophthalan
VC-13	*O*,2,4-dichlorophenyl *O,O*-diethyl phosphorothioate

trict in the field evaluation work in the Bakersfield area.

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A MAGNETIC METHOD OF SEPARATING MOSQUITO PUPAE FROM LARVAE

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Introduction. Mosquito larvae, in rearing cultures, do not all pupate uniformly, and the time from first to final pupation usually lasts several days, depending on crowding, food, temperature and some intrinsic characteristics of the larvae (Shannon and Putnam 1934, Bar-Zeev 1956). In some laboratories, the pupae are transferred every day or so, by means of pipettes into emergence cages (Trembley 1955, Christophers 1960). This method is time-consuming, and practical only when relatively small numbers of mosquitoes are required. In other laboratories one places the container with the larvae and pupae in or below an emergence cage, so that the mosquitoes after emergence enter the cage (McKiel 1957, Bar-Zeev and Galun 1960). This method has the disadvantage that the larvae which did not pupate in time are lost. Furthermore, the adults thus obtained are not of uniform age. A method of separating mosquito pupae from larvae may also be useful for experiments on sterilization by irradiation, since the irradiated stage is the pupa.

A mechanical device for separating mosquito pupae from larvae has been described by Fay and Morlan (1959). The method consists of pouring the culture, containing pupae and larvae, through two glass plates, slightly separated and of adjustable distance so that most of the pupae, being a little larger than the larvae, are retained between the glass plates, while the rest of the culture drains into a receiving container below. According to the authors the separation of 1,000 pupae requires about 20 minutes.

The principle of the present method is as follows: When separation of pupae and larvae is to be carried out, a little iron dust is added to the culture. The larvae ingest some of the iron particles, whereas the pupae (and prepupae) do not feed, and, therefore, do not contain any iron particles. Furthermore, larvae that feed, evacuate the contents of the alimentary canal before pupation, and thus free themselves again from the iron particles. The larvae and pupae are then exposed to a magnetic field, which will retain only the larvae.

Materials and Methods. *Aedes aegypti* L. larvae were reared in jars containing each about 1,500 larvae in about 3½ litres of water, and kept at a temperature of 28° C. Pupation started on the 5th day and the first adults emerged on the 7th day. On the 6th day, therefore, separation of the pupae was carried out. Two-tenths gr. of iron dust (hydrogen reduced iron, Merck & Co. Inc., Rahway, N. J.) were added to each jar and the mixture stirred for a few seconds with a stick. The culture was then left for about 30 minutes, during which time the larvae ingested enough iron particles for an efficient separation to take place. The larvae and pupae were then filtered through cheesecloth, freed from adhering iron particles by washing with water and placed in a jar ("magnetic jar"), made of metal or any other material (Fig. 1), filled with water to about one-third and fastened on top of a specially built electromagnet that can magnetize the bottom of the jar. The latter can be made of metal or any other non-magnetic material, since the magnet acts through the bottom of the jar. The larvae adhered to the bottom of the jar. Then the water with the pupae and prepupae was poured out by turning the apparatus upside down with the help of a lever. The apparatus was returned to its former position, the electric switch was turned off; some water was added to the jar and the larvae

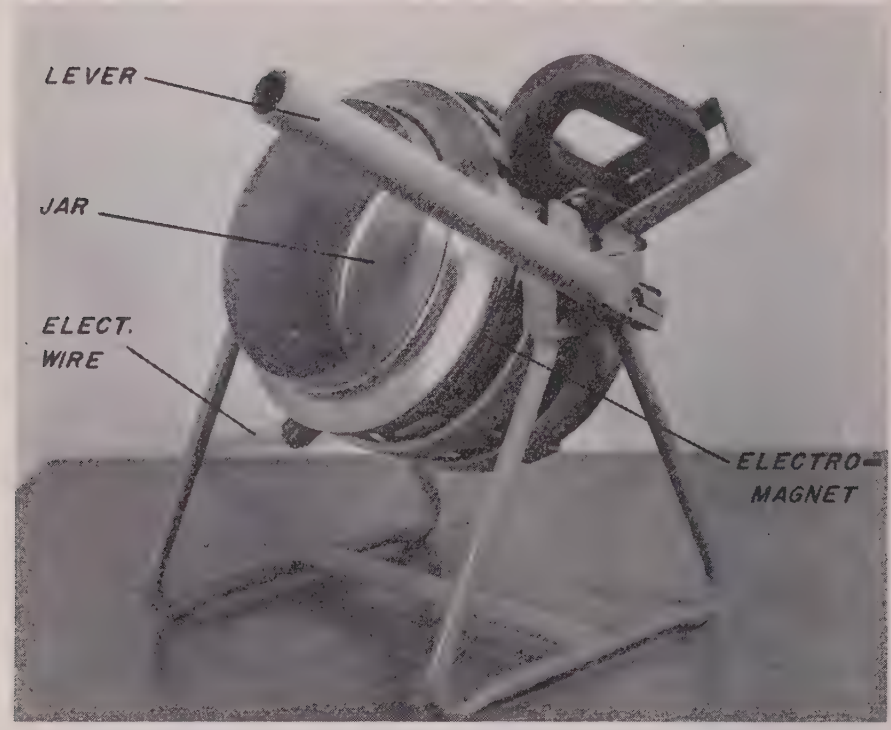

FIG. 1.—Apparatus for separating mosquito pupae from larvae

were poured into a prepared container.

A diagram of the apparatus is given in Fig. 2. The coil of the electromagnet is made of two parallel wires of 1 mm. in diameter and 820 turns. The current is 24 volts and 7 amperes. The larvae adhere to the bottom of the magnetic jar in two concentric circles, one corresponding to the circumference of the iron bar, and the other to the circumference of the metal plate (Fig. 2).

RESULTS. Most of the experiments were carried out with the mosquito *Aedes aegypti,* some with *Culex molestus* Forsk. Preliminary experiments with *A. aegypti* had shown that if too much iron is ingested by the larvae, their mortality and that of the resulting pupae is high, although those larvae and pupae which did survive produced healthy-appearing adults of normal life expectancy. Death seemed to be due to the clogging of the alimentary canal with iron particles. The amount of iron dust per breeding jar (about 1,500 *Aedes* larvae in 3½ litres of water) finally adopted was 0.2 gr., and the larvae were allowed to feed for periods of 20, 30 and 40 minutes.

After separation, the pupae include also some larvae, mostly prepupae, i.e. larvae which have stopped feeding and will pupate within 24 hours. Only a very small percentage of larvae (not prepupae) (Table 1), does not adhere strongly enough to the magnetic field and is found in the pupal group. The larval group contains very few pupae that remain stuck to the wall of the magnetic jar when pouring out the water containing the pupae; these pupae can, if desired, be removed by adding water once more to the jar and pouring it out.

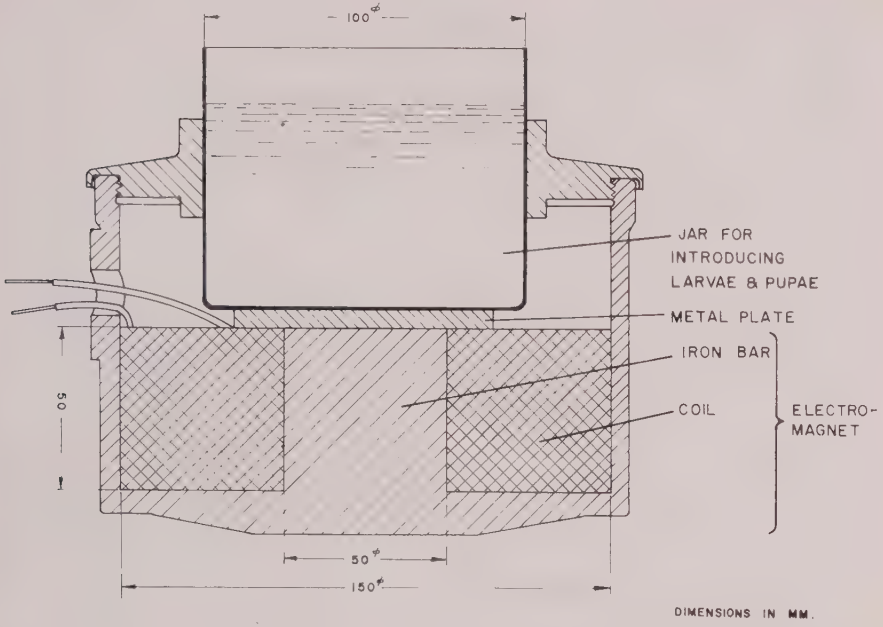

Fig. 2.—Diagram of the apparatus

Immediately after separation, the number of pupae and larvae in the pupal group and the number of pupae and larvae in the larval group was counted. Twenty-four hours after separation the number of larvae remaining in the pupal group (therefore not having been prepupae) was counted and the mortality of larvae and pupae in both groups recorded. (Larvae and pupae that died, as a result of the iron dust, did so within 24 hours.) Results are given in Table 1.

The separation of pupae was practically complete and the larvae in the pupal group were mainly prepupae; the mortality of larvae and pupae was negligible. In the larval groups, some food is added, and the separations of new pupae on the

TABLE 1.—Results of separating *A. aegypti* pupae from larvae *

	Duration of feeding (in min.)		
	20	30	40
Percent pupae separated ± S.E.	98.4±0.4	99.1±0.1	98.6±0.4
Percent of total larvae remaining in the pupal group after separation	15.3±1.0	11.8±3.6	4.2±0.4
Percent of total larvae remaining in the pupal group 24 hours after separation	1.1±0.4	1.9±0.3	0.7±0.2
Percent mortality of total larvae and pupae	2.1±0.5	1.7±0.6	2.8±0.7

* About 1,500 larvae and pupae per breeding jar of 3½ litres containing 0.2 gr. of iron dust (5 replicates).

following days are carried out in a similar manner, always with the same results.

Some separation experiments were also carried out with *Culex molestus*. It was found that under similar conditions of rearing, the amount of iron dust required to produce an effective separation was twice as much as for *A. aegypti*, viz. 0.4 gr. for the same period of time (30 min.). At this level, there was practically no mortality of *Culex* larvae and pupae; this mosquito seems, therefore, to be more resistant to the iron feeding. The reason for the larger amount of iron required by *Culex* may be the larger size of the larva.

In the method described, an ordinary horseshoe magnet of about 3 kg. strength may also be used, if relatively small numbers of mosquitoes are required. The magnet is covered with cheesecloth, and then submerged into the mixture of the larvae and pupae which had fed on the iron and had been filtered and placed in a small container with water. The magnet is moved back and forth in the water. The cheesecloth with the larvae adhering will be removed from the magnet and submerged in water to release the larvae. The same procedure is repeated several times until no more larvae adhere to the magnet.

DISCUSSION. The method used was found efficient with the two species of mosquitoes tested. It will most probably be effective also in other species of mosquitoes, since various species of mosquito larvae tested (Coggeshall 1926, Howland 1930a, 1930b) did not discriminate in the selection of food, and, therefore, will ingest the iron particles added to the culture. The amount of iron dust, and the time allowed to feed on it, will have to be determined for each species; it will also vary with the amount of water and larvae used per rearing container.

If too many larvae are separated at one time, the separation may not be as effective, since the whole magnetic surface at the bottom of the magnetic jar may be covered with adhering larvae. In such a case, the separation of the pupae (still containing laryae) should be repeated. It would be preferable to build the electromagnet so that the *whole* surface of the bottom of the magnetic jar will be strongly magnetized.

SUMMARY. A method is described by which mosquito pupae can be quickly separated from the larvae. Iron dust is added and the culture exposed to a magnetic field. Only the larvae which have ingested iron, will be affected by the magnetic field.

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THE STATUS OF INSECTICIDE RESISTANCE IN SOME MOSQUITO SPECIES OF TEXAS

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Introduction. Approximately four years after beginning the use of BHC (3 percent gamma isomer) as an adulticide in the Galveston County mosquito control program in 1956, salt-marsh mosquitoes in some parts of the county were noted by field personnel to be less affected by the insecticide than in previous years. It was especially noticeable that "barrier strips" did not provide population centers with as many days protection from flights of *Aedes sollicitans* as at the beginning of the program. More recently, there have been similar indications of increasing BHC-tolerance in *A. sollicitans* and *A. taeniorhynchus* in adjacent Brazoria County where the insecticide has been used intensively for a period of 2 years.

Prior to the above observations and before the organization of mosquito control districts along the Texas Gulf Coast, it was noted by mosquito control personnel engaged in the fogging of DDT in several communities of this area that *Culex fatigans* adults were largely unaffected by this insecticide. Likewise, populations of this species have not been controlled with BHC dust during recent years.

Approximately a year and a half ago, a study designed to determine the status of insecticide-resistance in certain mosquito species of Texas was begun, starting with those mentioned above (Micks, 1960). This work has been extended more recently to include *Culex salinarius,* which appeared in unusually large numbers in coastal areas during the first four months of 1961, replacing *C. fatigans* in many septic ditches. The results, representing the first report of resistance in mosquitoes of this state, are presented herein.

Materials and Methods. A number of different batches or strains of *Culex fatigans, Culex salinarius, Aedes sollicitans* and *Aedes taeniorhynchus* were collected at widely separated points in Galveston and Brazoria Counties. In most instances, sufficient larvae were collected to permit testing their susceptibility to DDT, gamma-BHC and dieldrin and to determine the susceptibility of the corresponding adults to DDT and dieldrin. In this way, a complete spectrum of resistance to chlorinated hydrocarbon insecticides was obtained.

Tests with larvae of the salt-marsh species were conducted using the water in which they were collected. Larvae of the two *Culex* species were tested in distilled water after it was determined that the use of the polluted water in which they were collected did not produce a significantly different test mortality.

At least two replicates of 25 to 30 fourth-stage larvae each were used for each insecticide concentration (0.004, 0.02, 0.1, 0.5 and 2.5 p.p.m.). Tests with adults utilized from three to five replicates, using 20 to 25 specimens per tube. The standard insecticide susceptibility test method of the World Health Organization was employed in each instance. However, if little or no mortality in adults occurred following 1-hour exposures to the highest insecticide concentrations, the exposure time was extended to 2 or more hours.

Results. The susceptibility to DDT of seven field-collected strains of *C. fatigans* larvae was compared with that of a susceptible strain (UTMB) and a DDT-resistant Okinawa strain (OK),

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both of which have been maintained in this laboratory for a number of years (Table 1). It can be readily seen that

TABLE 1.—Susceptibility of *Culex fatigans* larvae to DDT (p.p.m.)

Strain	.004	.02	.10	0.5	2.5
CLS	..	64	100	100	100
G	0	0	67	100	100
B	95	100	100	100	100
H	0	0	68	100	100
EC	0	10	70	100	100
A(59)	0	0	20	82	100
A(60)	0	0	29	100	100
UTMB	0	3	53	100	100
OK	..	0	0	19	57

all seven strains were susceptible to DDT and that two of them were considerably more so than the UTMB strain. On the other hand, Table 2 shows that two of

TABLE 2.—Susceptibility of *Culex fatigans* larvae to Dieldrin (p.p.m.)

Strain	.004	.02	.10	0.5	2.5
CLS	..	24	41	96	100
B	..	0	15	97	100
EC	..	0	8	84	94
A(59)	..	0	0	43	69
A(60)	..	0	0	11	53
UTMB	..	100	100	100	100
OK	..	8	28	54	79

these same strains, both from Angleton, Texas, were resistant to dieldrin.

All of the five strains of *C. fatigans* tested in the adult stage and which were susceptible to DDT as larvae, were found to be DDT-resistant (Table 3). Even the UTMB strain showed 15 percent survival upon 1-hour exposure. When exposure to 4 percent papers was extended beyond 1 hour, practically all specimens were knocked down; however, a considerable number of them recovered. All strains tested were found to be resistant to dieldrin (Table 4), the maximum mortality obtained in field-collected strains being only 7 percent following the customary 1-hour exposure period. Most

TABLE 3.—Mortality of adult female *Culex fatigans* after exposure to DDT (4%)

Strain	Hours exposure			
	1	2	3	24
EC	0	19	23	98
A(59)	10	..	88	100
A(60)	..	..	16	98
H	37	..	..	..
CLS	8	..	..	..
UTMB	85	..	..	..
OK	13	..	..	..

striking of all was the high level of dieldrin-resistance exhibited by the two strains from Angleton (A59 and A60), wherein 8 days of continuous exposure to 1.6 percent papers produced only 76 percent mortality in the latter.

Three strains each of *A. taeniorhynchus* and *A. sollicitans* larvae showed virtually complete susceptibility to DDT, dieldrin and gamma-BHC as exemplified by the BHC results shown in Table 5. On the other hand, several batches of *A. sollicitans* adults exhibited resistance to dieldrin (Table 6). Likewise, only 75 and 40 percent mortality occurred following 2-hour exposures of *A. taeniorhynchus* from two separate points in one locality.

More recently, *Culex salinarius* larvae from Brazoria and Galveston Counties were found to be completely susceptible to 0.02 p. p. m. of DDT, dieldrin and gamma-BHC. However, adults from these collections were resistant to dieldrin. One-hour exposures of two groups to 4 percent papers resulted in 26 and 27 percent mortality, respectively. In another test, only 30 percent were killed by a 6-hour exposure to 4 percent dieldrin. The mortality in four different strains of adults was 85, 71, 91, and 85 percent, respectively, upon 1-hour exposure to 4 percent DDT papers.

Table 7 summarizes the spectra of chlorinated hydrocarbon resistance in three species used in this study. Whereas all *C. fatigans* larvae were susceptible to DDT, those of the Angleton strains were dieldrin-resistant and the adults were re-

TABLE 4.—Mortality of adult female *Culex fatigans* after exposure to dieldrin

Strain	Hours exposure to 1.6%							
	1	2	3	5	16	18	24	192
EC	0	14	0	..	..	..	31	..
A(59)	7	..	..	80	..	70	..	..
A(60)	..	..	0	..	0	..	0	76
CLS	0	..	..	..	..	..	..	..
UTMB	19	..	..	..	..	..	..	..
OK	10	..	..	..	..	..	..	..

TABLE 5.—Susceptibility of *A. sollicitans* and *A. taeniorhynchus* larvae to BHC (p.p.m.)

Strain	.004	.02	.10	0.5	2.5
AT(Q) 7	100	100	100	100	100
AT(Q) 11	100	100	100	100	100
AT(C)	100	100	100	100	100
AS(C)	87	100	100	100	100
AS(VP)	93	100	100	100	100
AS(VP) 2	91	100	100	100	100

TABLE 6.—Mortality of adult female *Aedes sollicitans* and *A. taeniorhynchus* after exposure to dieldrin (1.6%)

Strain	1-hour	2-hour
AT(Q) 7	95, 92, 96	..
AT(Q) 11	..	75, 90, 40
AT(C)	95, 90, 90	..
AS(C)	50	..
AS(VP)	20	..
AS(SB)	100, 61, 58, 50	..

TABLE 7.—Patterns of susceptibility and resistance in larvae and corresponding adult females of *C. fatigans, C. salinarius,* and *A. sollicitans*

Species	Stage	DDT	Dieldrin
C. fatigans	Larvae	S*	R
	Adults	R	R
C. salinarius	Larvae	S	S
	Adults	S	R
A. sollicitans	Larvae	S	S
	Adults	S	R

* S=susceptible, R=resistant.

sistant to both insecticides. On the other hand, both *C. salinarius* and *A. sollicitans* larvae were susceptible to both insecticides, while the adults were DDT-susceptible and dieldrin-resistant.

DISCUSSION. The finding of resistance to both DDT and dieldrin in *C. fatigans* is not surprising since this species has shown insecticide-resistance in various parts of the world. DDT-resistance in *C. fatigans* was recognized in India in 1952 and was followed by Reunion, Taiwan, Okinawa, Venezuela, Puerto Rico, South Australia, Panama, Hawaii and the Congo, during the ensuing seven years. Resistance to BHC and/or dieldrin was reported from California in 1951 and is also present in Malaya, India, Asia, South America, West Africa, Panama, Zanzibar and the Congo. In 1959, malathion resistance in this species at Duala, Cameroun was reported (Brown, 1961).

Whereas in other parts of the world, dieldrin-resistance has been found in adult populations where residual applications of this insecticide have been used for several years, dieldrin-resistance in the United States has frequently occurred in the apparent absence of the use of this insecticide.

The finding of dieldrin-resistant *C. salinarius* in Texas represents the first report of insecticide-resistance in this species.

The development of DDT-resistance in *A. sollicitans* and *A. taeniorhynchus* in Florida in 1947 and 1949, respectively, was followed several years later by resistance to the BHC-dieldrin group and more recently by malathion-resistance (Brown, 1961). *Aedes sollicitans* has also become resistant to DDT (Darsie *et al.,* 1957) and BHC (Darsie and Sutherland, 1959) in Delaware. Dieldrin-resistance was demonstrated in *A. taeniorhynchus*

from a small focus in Chatham County, Georgia, and was accompanied by a degree of cross-resistance to DDT (Schoof, 1959).

It appears likely that the dieldrin-resistance exhibited by the several species used in this study is largely a result of the use of BHC in mosquito control programs. On the other hand, the rapidity of development of dieldrin-resistance in species from Brazoria County might be partly accounted for by the use of aldrin in connection with extensive rice-growing areas.

Since the use of DDT in these two counties has been confined to the infrequent fogging of only a few communities, the agricultural use of the insecticide may have contributed to the widespread DDT-resistance in *C. fatigans*.

One of the important points to emerge from this study is that insecticide susceptibility test results from the larvae alone can be very misleading and should, therefore, be accompanied by data from the corresponding adults. Although the adults of all species studied were resistant to dieldrin, the larvae, with the exception of two strains did not exhibit resistance.

SUMMARY. Adult *Culex fatigans, C. salinarius* and *Aedes sollicitans* reared from larvae collected from widely separated localities in Galveston County and Brazoria County, Texas, exhibited resistance to dieldrin. DDT-resistance was also present in *C. fatigans*. Except for two strains of *C. fatigans,* the larvae of this species as well as those of *C. salinarius, A. sollicitans* and *A. taeniorhynchus* were susceptible to DDT, dieldrin and BHC.

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DDT-RESISTANCE IN A STRAIN OF *CULEX PIPIENS* FROM NORTHERN ILLINOIS

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The South Cook County Mosquito Abatement District comprises 340 square miles in the south part of Cook County, Illinois. The District includes approximately all of the area south of 87th Street in the City of Chicago to the Cook-Will County line in the south and west and the Indiana state border to the east. DDT has been the principal insecticide used by the District during the past six years.

During the 1960 mosquito season, several reports were received from field personnel that they could not achieve control of *Culex pipiens* in certain areas of the District with their routine larviciding dosage rate of 0.2 pound of DDT per acre. Formerly, good control had been possible at this rate of application.

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In order to ascertain if this control failure was due to resistance, it was decided to employ the standard World Health Organization method for determining the susceptibility or resistance of mosquito larvae to insecticides. A laboratory colony of *C. pipiens* was established from numerous larvae and pupae collected from one of the locations in which control difficulty had been reported. The area has a history of heavy exposure to DDT for the past five years. This colony has been named the "Midlothian Creek Strain," and the larvae used in this particular work were taken from the colony when it was in its 8th to 10th laboratory generation. It is believed this strain is made up of the subspecies *C. pipiens pipiens,* as numerous attempts to induce oviposition without a blood meal have ended in failure.

Table 1 indicates the results of four replicated experiments which were performed with the WHO kit and according to the instructions contained therein.

TABLE 1.—Susceptibility of Midlothian Creek Strain of *C. pipiens* to DDT

DDT concentration (p.p.m.)	Total larvae	Moribund and dead	Percentage mortality
.004	150	0	0
.02	200	1	0.5
.10	200	18	9.0
.25	50	23	46.0
.5	200	151	75.5
2.5	200	200	100.0
Control	200	0	0

After plotting the percentage mortalities on logarithmic probability paper a dosage-mortality regression line was drawn, and the LC-50 value was determined to be 0.28 p.p.m. DDT.

In the fall of 1960 it was possible to perform two replicated tests on *pipiens* larvae collected in the field in another part of the District. This particular area had received considerably less DDT during the past five years than the Midlothian Creek location. An LC-50 value of .025 p.p.m. DDT was determined for this population, but this figure is based on the results of only two replicated experiments instead of four.

Burbutis and Davis (1955) found an LC-50 value of 0.2 p.p.m. DDT for a New Jersey strain taken from an area in which DDT had been used for five years. They also obtained an LC-50 of 0.013 p.p.m. DDT for another New Jersey strain of this species taken from an area in which no DDT had been used. Brown, *et al.* (1954) found a value of 0.02 p.p.m. DDT for this species in an area in southern Ontario that had a history of slight exposure to DDT.

Because of the significant difference in the level of susceptibility of the Midlothian Creek Strain as compared to the other strain from the District, the non-resistant strain from New Jersey, and the population tested in Canada, it is the opinion of the writers that the term "DDT-Resistant" should be applied to it.

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THE SIGNIFICANCE OF WATER-HOLDING CAVITIES OF TREES AS MOSQUITO FOCI WITH SPECIAL REFERENCE TO *AEDES AEGYPTI* CONTROL PROGRAMS

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Entomologists have long exhibited interest in the public health significance of mosquitoes breeding in tree holes. Jenkins and Carpenter (1946) reported on the habits and distribution of eight species and one subspecies of mosquito which usually breed only in this habitat. Among these species they recorded that malaria has been carried experimentally by *Anopheles barberi* Coq. and yellow fever by *Aedes triseriatus* (Say). The distribution of *Aedes triseriatus* corresponded well with a 1941 outbreak of eastern encephalomyelitis in Massachusetts. Bonnet and Chapman (1956) reported upon the importance of the tree-hole-breeding mosquito, *Aedes polynesiensis* Marks, with reference to filariasis in Tahiti. Shute (1954) indicates that indigenous malaria in England may be transmitted by tree-hole-breeding *Anopheles plumbeus* Stephens. The threatened introduction of yellow fever from South and Central America, coupled with the recovery in 1955 of *Haemagogus equinus* Theobald larvae from tree holes in the vicinity of Brownsville, Texas, (Trapido and Galindo 1956; Eads and Strom 1957) has served to intensify the examination of such cavities in and around airports and dock areas receiving international traffic in the southern United States.

In the conduct of yellow fever control programs it is desirable to have all vestiges of *Aedes aegypti* breeding eliminated, otherwise the program becomes one of constant surveillance with the possibility of continuous reinfestation from hidden sources. Tree holes, frequently so minute as to be easily overlooked, can harbor these mosquitoes.

The *Aedes aegypti* Detection and Control Units assigned to the Brownsville, Texas; Miami Beach, Florida and New Orleans, Louisiana, Public Health Service Quarantine Stations have been actively searching since late 1957 to determine the importance of *aegypti* breeding in various tree holes in these locations.

Although species known to associate with the yellow fever mosquito were recovered in all three areas, *aegypti* larvae have been encountered frequently in these tree hole surveys in south Florida only. A total of 2237 tree hole inspections have been made in this region between 1958–1961; 99 of these inspections were positive for *aegypti*. In one particular area where 380 premises are routinely inspected, 24 of these premises had one or more tree holes that were inspected. Thirteen of these premises were positive for *aegypti*. Thus, while all the artificial containers might be treated for *aegypti* breeding on these premises, there would still be an *aegypti* index of 3.4 percent (13 positive tree holes ÷ 380 premises examined). These untreated tree holes could then serve as foci to reinfest the treated areas.

A number of species of trees in urban areas have been examined. Table 1 lists the trees and the species of mosquitoes recovered from them. It is apparent that the species of trees involved does not necessarily dictate the species of mosquitoes to be found. It does, perhaps, indicate that certain trees lend themselves more readily to forming cavities and rot

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TABLE 1.—Species of trees containing rot-holes, depressions and cavities and the species of mosquito larvae recovered from them[1]

Species of Trees	*Aedes aegypti*	*A. triseriatus*	*A. zoosophus*	*Culex nigripalpus*	*C. pipiens-quinquefasciatus*	*C. restuans*	*Deinoceries cancer*	*Haemagogus equinis*	*Orthopodomyia alba*	*O. signifera*	*Toxorhynchites r. rutilis*	*T. rutilis septentrionalis*
Avicenna nitida (Black mangrove)	..	x	..	..	..	..	..	..	..	..	..	..
Bambusa sp. (Bamboo)	..	x	..	..	x	..	..	..	..	..	..	..
Bougainvillaea (Bougainvillea)	..	x	..	..	..	..	..	..	..	..	..	..
Casuarina sp. (Australian pine)	..	..	..	x	..	..	..	..	..	..	..	..
Celtis spp. (Hackberry)	..	x	x	..	..	..	..	..	..	x	..	..
Cornus florida (Flowering dogwood)	..	..	..	..	..	..	..	..	..	x	..	x
Diospyros texana (Chapote)	..	..	..	..	..	..	..	..	..	..	..	x
Ficus spp. (Banyan & Strangler fig)	x	x	..	x	x	..	x	..	..	x	..	..
Kigelia pinnata (Sausage tree)	x	..	..	..	..	..	..	..	..	..	..	..
Lagerstoemia indica (Crepe myrtle)	..	x	..	..	..	..	..	..	..	..	..	..
Pithecolobium flexicaule (Texas ebony)	..	x	x	..	..	..	..	x	..	..	..	..
Poinciana regia (Royal poinciana)	x	x	..	..	x	..	..	..	..	x	..	..
Pongamia pinnata (Pongam, Poonga Oil Tree)	x	x	..	..	..	..	..	..	..	..	..	..
Prosopis sp. (Mesquite)	..	x	..	..	..	..	..	..	..	..	..	..
Quercus spp. (Oaks)	x	x	x	..	x	x	..	..	x	x	x	x
Salix sp. (Willow)	..	x	x	..	..	..	..	..	..	..	..	..
Sapindus sp. (Chinaberry)	..	x	x	..	..	..	..	..	..	..	..	..
Ulmus sp. (Elm)	..	..	..	..	..	..	..	..	..	x	..	..

[1] Based on data from the *Aedes aegypti* Detection and Control Units of the Brownsville, Texas, Miami Beach, Florida and New Orleans, Louisiana, Quarantine Stations.

hole conditions that will hold water and thus make them more attractive for breeding.

It is desirable to be familiar with these frequently-hidden sources of breeding. Recognition of the "offender" is only part of the battle. Action must be taken. Ordinarily the domestic mosquito control man is not prepared for an extensive tree hole mosquito control project. Robinson (1959) recently reported treating 4500 tree holes in one month in Alameda County, California, raising his winter-season tree hole treatment to 22,936. Undoubtedly many areas in the sub-tropics and tropics could duplicate these results in the event of a thorough survey. Since trees in sub-tropical parts of the United States present an abundance of mosquito-breeding cavities several avenues of approach may be taken. Public Health Service Quarantine personnel have routinely made monthly tree hole surveys, inspecting many trees in the areas of coverage. Records are kept of the location, the species of trees involved, the presence or absence of water and/or mosquito larvae and pupae.

Different conditions will dictate different methods of control. Control measures for tree hole mosquitoes used in the past and those that might be useful in the future are as follows:

I. PERMANENT CONTROL MEASURES:

A. Cultural Procedures:

1. Removal of offending trees and replacement with species less apt to have, or develop, water-holding cavities.
2. Pruning of trees to remove cavities.

3. Insertion of drains into cavities.
4. Filling of cavities with cement or tar.
5. Relocation of sprinkler heads.
6. Topping cavities with living plants.

Of these measures, because of the nature of the most common offender in south Florida (the various species of *Ficus,* i.e., strangler fig and banyan) periodic pruning would be required to prevent formation of new water-holding cavities. In most cases it is not feasible to attempt to locate and fill all water-retaining areas in trees. Improperly placed lawn sprinkler devices are surprisingly common conditions. A word spoken to the owner of the property, where lawn sprinkler devices keep tree holes perpetually wet and breeding mosquitoes, is often sufficient to obtain the necessary changes. Except in the limited areas near places of international traffic, hand methods of eliminating tree holes by cultural procedures are probably not feasible in the United States. The education and encouragement of property owners to treat their own trees may be a practical approach. A novel approach requires partial filling of cavities with dirt and gravel and placing a living plant, usually a fern, on top.

B. Biological Measures:

The practical use of predaceous *Toxorhynchites* mosquitoes has not been determined for this country. These mosquitoes have, however, proved of little value in the Hawaiian Islands, (Bonnet and Hu 1951.)

II. TEMPORARY CONTROL MEASURES:

A. Sand or earth fills of tree hole cavities:

B. Insecticides:

1. *Hand applications.*

Since *aegypti* have been found breeding in tree holes from near ground level to 34 feet (Kellet and Omardeen, 1957) the hand application of insecticides would be difficult and expensive. In areas where many small containers breeding *aegypti* have been involved, such as cemetery urns and tree holes, use has been made of various types of insecticidal briquettes that would gradually give off their toxicant and provide control over a longer period of time. Kellet and Omardeen used briquettes of benzene hexachloride in plaster of paris for *aegypti* breeding in tree holes in Trinidad. Le Van (1940) used paris green with plaster of paris for *aegypti* breeding in cemetery urns in Key West, Florida. The application of dieldrin-cement pellets will prevent breeding of *aegypti* in containers up to a year, (Elliott, 1955 and Public Health Service, 1961). Briquettes of these same ingredients were effective in maintaining fire barrels free of reinfestation for a five-month inspectional period (Evans and Fink, 1960). One of the authors (JEP) has maintained mosquito free conditions in tree holes with dieldrin-cement briquettes for over eight months at Miami, Florida.

2. *Power application.*

The use of power machinery in the application of insecticides has become increasingly popular in the United States because it enables a few men to cover a large area with insecticide at a minimum cost. However, it is doubtful if aerosols would occur in high enough concentration to be toxic to tree hole larvae.

Larger insecticidal particles would have difficulty in penetrating the leaf foliage whether applied from the ground or the air. Perhaps a fine dust might be satisfactory. Certainly more consideration should be given to control of mosquitoes breeding in tree holes if successful *aegypti* eradication is to be accomplished.

Acknowledgments

The field assistance of Martin J. Kuck, Biologist, and Richard J. Peck, former Biologist, with the Miami Quarantine Station and of Edward J. Fink, Biologist, New Orleans Quarantine Station is gratefully acknowledged.

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UTAH MOSQUITO ABATEMENT ASSOCIATION

Sixty per cent of the people in the state of Utah are now living within the boundaries of organized mosquito abatement districts.

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FIFTEENTH ANNUAL MEETING
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OBSERVATIONS ON THE BITING AND SWARMING OF SNIPE FLIES (DIPTERA: SYMPHOROMYIA) IN THE FOOTHILLS OF SOUTHERN ALBERTA [1]

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INTRODUCTION. During the summer of 1959 the authors were attacked severely by snipe flies while working in the foothills west of Claresholm, Alberta. A few specimens of the flies were first identified as *Symphoromyia hirta* Johnson (Brooks, 1959) but an expanded series was later re-appraised by Chillcott (1960) with reference to the Canadian National Collection in Ottawa. Their exact taxonomic status was found to be uncertain beyond membership in a *Symphoromyia hirta* complex, but for simplicity they will be referred to as *S. hirta* in this paper.

Reports of *S. hirta* activity on the eastern slopes of the Rocky Mountains in the United States (Knowlton and Maddock 1944, Mills 1943, and Stanford 1931) include descriptions of severe attacks on man and animals. There have been no published reports of attack by this species in Canada, but *S. atripes* Bigot has been collected on the western slopes of the Rocky Mountains in Canada (Hearle 1928), the United States (Ross 1940), and Alaska (Frohne 1959, Frohne and Williams 1951, Sailer 1951).

The only record in our laboratory contained a report of fly attack and a small number of specimens of *S. hirta* from Burmis, Alberta in 1955. However, inhabitants and tourists in the area were sufficiently familiar with the pest to warrant its consideration as a severe, if intermittent, nuisance.

OBSERVATIONS AND DISCUSSION. Most of the observations were conducted in 1959 and 1960 on a knoll (Fig. 1) of a wooded ridge, which ran generally in a southwesterly direction, in the Porcupine Hills about 25 miles west of Claresholm, Alberta. The main observation site was located on the crest of the ridge and on the approaching slopes at an altitude of approximately 5,200 ft. above sea level. The vegetation consisted predominately of scrub Douglas fir, *Pseudotsuga taxifolia* Britt. var. *glauca* (Mayr.) Sudw. with occasional black spruce, *Picea mariana* (Mill.), scrub pine, *Pinus albicaulis* Engelm., aspen, *Populus tremuloides* Michx., and an undergrowth of juniper, *Juniperus scopulorum* Sarg., cow parsnip, *Heracleum lanatum* Michx., other forbs, and native grasses (Fig. 2). This ridge was utilized as a late summer range for cattle and contained numerous well-beaten trails among the trees. Observations were also taken around a parked automobile on the open range below this ridge, around herds of cattle and some horses. In addition we observed fly activity around ranch buildings 3 miles away and 1,000 feet lower; these were in a poplar-spruce grove adjacent to a small stream. Spot checks were also made along the foothills from Pincher Creek to Stavely at sites that were similar to our main observation area.

Only the female flies bit and their attack was somewhat similar to that of deer flies (*Chrysops* spp.) in that they landed unobtrusively and inflicted bites before their presence was realized. Unlike deerflies they gathered in "following swarms" about the head and shoulders and their intense hum was characteristic of much larger insects. They also crawled under shirt cuffs and collars, and under trouser legs to bite. The disproportionately loud hum and the "crawly" feeling tended to induce panic. The flies bit as readily on the tough palm of the hand as on the

[1] Contribution from the Veterinary-Medical Entomology Section.

Fig. 1.—A general view of the main snipe fly observation site in the Foothills of southern Alberta

Fig. 2.—A pathway through the woods along which male swarms were found

more tender skin of the face and the back of the neck (Fig. 3).

When unmolested they probed for blood several times, each probe forming a new puncture. Blood usually oozed for a while after the probing, which may

FIG. 3.—Erythematous macropapules on the back of the neck and the palm of hand three days after being bitten by snipe flies

be due to an anticoagulant present in the salivary secretions and/or to the severe laceration from the numerous probes in the one location. The bite was keen and painful unlike the dull sting of a mosquito. An erythematous macropapular reaction set in almost immediately and persisted with accompanying irritation for 3–4 days. Victims reacted variably: of the four observers working in the same location at the same time, one reacted violently showing severe inflammation (Fig. 3), two had moderate inflammation, and one appeared to be unaffected. One of the moderately-reacting observers was the most panic-stricken. He draped an insect net over his head, slapped frantically at the assembled flies, and finally had to leave the observation area.

In 1959, relief from the flies could be obtained by leaving the brush and going to the open prairie. Conversely, a person was not bothered until he approached within a few hundred feet of trees and shrubs. The numbers of flies that were attracted increased as one went deeper into the brush, but did not decrease appreciably in the small openings among the trees. However, in 1960 the flies attacked the observers and ranchers in hay fields about 5 miles away from the wooded areas. Large numbers of the flies followed a farmer operating a tractor in an open hay field, and another man who was engaged in haying was so affected by their bites as to be bedridden for a few days. The flies were attracted to a light green car parked on the open prairie downhill from observation site in moderate numbers in 1959, and in much greater numbers in 1960.

Livestock generally did not seem to attract large numbers of the flies but some yearling and calf steers were bothered by an average of about 15–30 flies. One tethered calf in the brush was observed closely and the flies congregated mainly about the head and to a lesser degree about a raw brand from which they were sucking oozing blood. These cattle were either on the lee side of a brushy area or in the brush itself. Another herd of yearling cattle, in the open and subject to high winds, were completely devoid of snipe flies, though attacked by horse flies, deer flies and horn flies. Three horses, examined in the direct sunlight in 1959 and in the shade in 1960, attracted no snipe flies. Both times, however, they were on the windward side of buildings. Hearle (1938) reported that *Symphoromyia* spp. attacked pack horses and sheep in the higher plains of British Columbia.

The seasonal distribution of the flies began in late June, reached a peak about mid-July and declined by mid-August. The greatest concentrations of flies seemed to be in poplar and conifer stands in the foothills observation station, but they were also plentiful around the ranch buildings and surrounding trees. It was first thought that the population in the wooded areas was somewhat different in behaviour from the population in the open areas. Although both were persistent in their attack, the population from the wooded areas followed observers out into the open for only a few yards and ceased to be a nuisance, whereas the population on the open prairie pursued the observer into the shaded or wooded area. These differences seemed to be based on wind intensity, because the flies that attacked in the open did so when the wind speed was less than 500 ft./min.

Residents of the Claresholm district stated that this pest had not been as serious as in 1959 and 1960 for a considerable time. Residents of the foothills recalled that previous serious outbreaks occurred in 1948 and 1954. These flies were given such names as "Sleepy flies" and "Lazy flies" but were sometimes confused with blackflies, stable flies, horn flies and house flies. Additional observations and reports from as far south as Waterton Lakes and as far north as Valley View indicated that the outbreak was not localized. *S. hirta* has been also reported as seriously annoying man and cattle in Utah for the years 1958 and 1960 (U.S.D.A. 1958, 1960).

Males of this species were plentiful at

the main observation site, but they exhibited no interest in human beings and appeared to be confined to typical male swarms. Large swarms up to a length of 100 yards formed along certain pathways (Fig. 2) through the trees and in aggregation resembled "marker swarms" as described for mosquitoes by Nielsen and Haeger (1960). However, the flight of individuals was noticeably more erratic than the flight formations taken up by swarming male mosquitoes and chironomids.

Females were abundant in the vicinity of these male swarms and entered the swarms in a ratio of 1 female to 5 males, a higher ratio than that observed for mosquitoes. These swarming sites were the only occasions when both sexes were caught together. It is possible that they represented mating swarms, even though actual copulation was not observed. The swarms first formed at about 7:30 a.m. and continued until late afternoon depending on the weather conditions. Orientation of the males appeared to be predominantly into a slight wind and at right angles to the sun. Swarms moved higher as the time of day progressed, probably in response to the increasing height of the sun's rays through the trees. On sunny days swarming was vigorous and could be detected by a characteristic hum, but activity was reduced when clouds obscured the sun even momentarily. No swarms were observed on cloudy cool days. Sweeping through the swarm with an insect net dispersed the insects and it took one or two minutes for them to regroup. This is the first known report of male swarming for *S. hirta*. A previous report of vertical swarms above water hydrants (Knowlton and Maddock 1944) did not identify the sex of the swarming flies, but the context of the report suggested that they were females.

Nothing is known of the breeding places and the life history of the species. The abundance of adults would indicate that larvae and pupae should be present in the vicinity of the foothills. In the early spring of 1960 a search for larvae and pupae was made in rotting sawdust and decaying organic matter along streams and in soil around permanent sloughs in the vicinity of the observation sites, but none were found.

Snipe flies are of potential nuisance value to the increasing number of tourists that frequent the area as well as to the resident farmers and lumbermen. Apart from the nuisance value, their persistent attack, along with the many probes made in the process of obtaining a blood meal, might make them a possible mechanical vector of disease. The labella are similar to those of *S. atripes* as described by Ross (1940), being large and possessing well-developed pseudotracheal system and numerous bristles. This feature might enhance its potential as a disease vector.

Control of this pest will be difficult until more knowledge is obtained on the life-history and habits. Without this information the use of repellents would seem to be the best way to protect humans. Rutger's "612" was tried but it proved ineffective against these flies. No organized attempt has been made to protect livestock against snipe flies in the field. Pyrenone sprays were unsuccessful in reducing the number of flies annoying a pet baby calf on the ranch near the observation station.

SUMMARY. *Symphoromyia hirta* was found to be a serious pest of humans in the foothills of Alberta during the summers of 1959 and 1960. The females inflicted bites on exposed parts of the body. The bites were very painful and produced an almost immediate erythematous macropapular reaction, which persisted with accompanying irritation for about three to four days. Males did not attempt to bite. Livestock were attacked in moderate numbers.

The flies were most abundant in the wooded areas at an elevation of approximately 5,200 feet, but females were also found around farmsteads and open pastures at lower elevations of approximately 4,200 feet. Male swarms, which are believed to be mating swarms, formed up

along certain pathways in the brush at about 7:30 a.m. and were present until early afternoon, with a gradual movement upward as the time of day progressed. The males in the swarm were orientated into a slight wind and at right angles to the sun. The ratio of the sexes in the swarms was 1 female to 5 males. Overcast skies inhibited this swarming activity. Strong winds prevented activity of both males and females.

Acknowledgments

The authors wish to express thanks to Mr. A. R. Brooks, Canada Agriculture Research Station, Saskatoon, and Dr. J. G. Chillcott, Entomology Research Institute, Ottawa, for identifying the specimens; to Mr. E. T. Gushul, photographer, for being such a good reactor to the bites, to Mr. R. Schweitzer, student assistant, and Mr. M. C. Qually, technician, for assistance in making the observations.

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WITH APOLOGIES: The photographs on page 146 of our June number (Vol. 21, No. 2) were taken by Tommy Mulhern. This credit line should have appeared with the photographs.

ISOLATION OF EASTERN ENCEPHALITIS VIRUS FROM DIPTERA IN WISCONSIN[1]

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INTRODUCTION. The isolation of eastern encephalitis virus from a turkey and the serological evidence of widespread infection among turkeys in southwestern Wisconsin in 1959 (Spalatin, *et al.*, 1960) stimulated a study of EEV transmission among turkeys in 1960. In 1959, attempts were not made to isolate virus from arthropods until after serological results were known. Therefore, only 137 Diptera of 9 species were tested, all with negative results.

Turkey sera were again examined for neutralizing antibodies to both EEV and WEV in 1960, and increased emphasis was given to collecting and processing hematophagous Diptera from areas of known virus activity in the previous year. In the 1960 search for naturally infected blood-sucking flies, 402 pools of 10,635 specimens representing 32 species were processed in laboratory hosts.

MATERIALS AND METHODS. Throughout May and June, flies were collected from the windows of brooder houses containing young poults, and some were occasionally aspirated directly from birds. Field collections were made from April through September by exposing caged birds and capturing all insects attracted to and feeding on them as described by Anderson and DeFoliart (1961).

On most occasions living flies were transported to the laboratory where they either were anesthetized or killed by exposure to a temperature of —20 C. for 5 to 30 minutes. On a few occasions flies were immobilized by chloroform in the field, rapidly transferred to screw cap vials, placed in a thermos bottle containing dry ice, and transported to the laboratory. All flies were identified in the laboratory and pooled by species for virus isolation attempts. Depending on the number of insects collected on a specific date and the availability of laboratory hosts, the insect pools varied in size from one specimen to 116 of a species. Engorged and non-engorged specimens of a species were usually processed in separate pools.

Insects were triturated in 1 to 3 ml of tryptone broth, normal calf sera, or 20% antibody-free egg yolk (pH 7.7) containing 5000 units of penicillin and 5 mg of dihydrostreptomycin per ml. After large particulate matter had settled out, the supernatant fluid was filtered through sterile cotton and 0.10 ml inoculated into the allantoic chamber of 10-day-old embryonated eggs or subcutaneously into ½-day-old chicks. The brains of chicks which died and fluids from dead embryos were tested for bacterial contamination on nutrient agar and in thioglycollate broth. Bacteria-free harvests were tested for Newcastle disease virus and then repassed in either the above hosts or 3-day-old suckling mice. Mice received 0.02 ml of the inoculum intracerebrally and 0.03 ml intraperitoneally. Isolates were identified by standard virus neutralization tests in 10-day-old chick embryos.

Blood was collected from the experimental hosts at weekly or bi-weekly intervals prior to and after their exposure in the field, and examined for the presence of *Leucocytozoon* parasites and neutralizing antibodies to both EEV and WEV. As

[1] Contribution from the Departments of Veterinary Science and Entomology. Approved for publication by the director of the Wisconsin Agricultural Experiment Station. This research was supported in part by a Public Health Service Research Fellowship (EF-10,649) from the National Institute of Allergy and Infectious Disease, and in part by a Public Health Traineeship (60–309).

TABLE 1.—Summary of virus isolations, 1960

	Modified passage history[a]								
	First			Final				Log Neut. index	
	Host[b]	Mortality[c] (No.)	Incubation (Hrs.)	Host	Mortality (No.)	Incubation (Hrs.)	Titration[d]	EEV	WEV
Eusimulium johannseni (Hart)	CE	2/12	72–120	SM	9/9	24–72	7.0	1.8	0
Aedes sticticus (Mg.) [e]	CE	5/12	48–120	SM	11/11	24–56	7.2	2.3	0
Simulium meridionale Riley	CE	5/12	98–144	WC	4/6	24–48	5.5	2.7	0
Aedes sticticus (Mg.)	WC	2/6	24–96	CE	6/6	24	3.7	1.7	0

[a] Different isolates underwent from 2–5 passages before identification.
[b] CE=10-day-old chicken embryo; WC=one-half-day-old chicks; and SM=3-day-old suckling mice.
[c] Number dead over total number inoculated.
[d] Titered from final passage material in 10-day-old chicken embryos.
[e] Collected in turkey brooder house on June 8.

in 1959, blood was also obtained from several turkey flocks in the study area. Serum for antibody detection was collected on absorbent paper discs and tested as an eluate in the manner described by Adams and Hanson (1956) and Karstad, *et al.*, (1957).

RESULTS. Four isolations of EEV were made from the 402 pools of flies processed in 1960. These isolates were from a pool of 46 engorged *Eusimulium johannseni* (Hart) which had fed on an experimental turkey poult and a ring-necked pheasant exposed on May 2; a pool of 15 engorged *Aedes sticticus* (Mg.) and a pool of 100 non-engorged *Simulium meridionale* Riley, both pools collected in a turkey brooder house on June 8; and a pool of 5 engorged and 2 non-engorged *Ae. sticticus* collected from pheasants exposed as experimental field hosts on August 4. The isolation histories are summarized in Table 1. This is believed to be the first report of EEV being isolated from these species in nature.

Hematophagous Diptera processed for chicken embryo or wet-chick lethal agents included 8,464 Simuliidae, 1,122 Culicidae, and 1,049 Ceratopogonidae, classified as follows. The figures are the total number of pools inoculated, over the total number of individuals processed, per species.

SIMULIIDAE: *Cnephia taeniatifrons* (End.)—9/82; *Eusimulium aureum* (Fries)—25/255; *E. johannseni* (Hart)—57/750; *Simulium rugglesi* N. & M—2/146; *S. meridionale* Riley—158/7,236; *S. venustum* Say—1/10, and *S. decorum* Walk.—1/15. CULICIDAE: *Aedes cinereus* (Mg.)—3/5; *Ae. excrucians* (Walk.)—4/26; *Ae. sticticus* (Mg.)—14/169; *Ae. stimulans* (Walk.)—8/78; *Ae. triseriatus* (Say)—2/5; *Ae. trivittatus* (Coq.)—7/133; *Ae. vexans* (Mg.)—23/473; *Anopheles punctipennis* Say—6/79; *An. quadrimaculatus* Say—3/14; *An. walkeri* Theobald—5/51; *Culex erraticus* (D. & K.)—1/3; *C. pipiens* L.—4/8; *C. salinarius* Coq.—1/2; *C. tarsalis* Coq.—1/2; *C. territans* Walk.—1/1; *Mansonia perturbans* (Walk.)—7/41; Mixed species—4/35. CERATOPOGONIDAE: *Culicoides arboricola* R. & H.—4/10; *C. biguttatus* (Coq.)—5/240; *C. crepuscularis* Mall.—18/513; *C. guttipennis* (Coq.)—1/4; *C. haematopotus* (Mall.)—14/173; *C. stellifer* (Coq.)—2/15; *C. variipennis* (Coq.)—1/12; *C. venustus* Hoff.—2/71; *C. villosipennis* (R. & H.)—1/3; Mixed species—1/8.

In Table 2, insect genera are tabulated in the monthly sequence in which they were captured and processed. The April total represents blackflies collected from 4 experimental hosts exposed in a woodland habitat near a turkey farm during the last week of April. At this time the principal blood-sucking fly collected was *Cnephia*

TABLE 2.—Genera of Diptera captured and inoculated into laboratory hosts by month, 1960. (Figures are the total number of pools inoculated per genus over the total numbers of the several species.)

Genera	April	May	June	July	August	September	No. pools	No. individuals
Cnephia	5/50	4/32	..	..	..	..	9	82
Eusimulium	1/1	56/749	1/7	4/44	16/133	4/41	82	975
Simulium	..	40/2099	22/1661	12/420	74/2705	14/522	162	7407
Culicoides	..	..	3/124	7/168	34/720	5/37	49	1049
Aedes	..	8/109	18/170	10/64	33/515	2/76	71	934
Anopheles	..	..	1/3	..	9/73	3/62	13	138
Mansonia	..	..	..	1/6	6/26	..	7	32
Culex	..	..	..	1/1	7/15	..	8	16
No. pools	6	108	45	35	179	28	401[a]	..
No. individuals	51	2989	1965	703	4187	738	..	10,633[a]

[a] A mixed pool of one *An. walkeri* and one *C. pipiens* are excluded from the totals. Grand total= 402 pools and 10,635 individuals.

taeniatifrons. This fly was not found in brooder houses, but it was attracted to and fed on turkeys exposed in the field (Anderson and DeFoliart, 1961).

The May and June totals include both brooder house collections and flies captured after being attracted to experimental host birds in the field. All July, August, and September totals are from experimentally exposed hosts. Brooder house visits were made on April 23 and 29, May 2, 7, 14, 21, and 31, and on June 8. After the latter date all farm turkey flocks were on open ranges. No hematophagous insects were observed in the houses prior to May 7.

The following species were represented in the various genera collected during May: *C. taeniatifrons, E. johannseni, S. meridionale, Ae. cinereus, Ae. vexans, Ae. sticticus,* and *Ae. stimulans*. All mosquitoes and the 1,953 *S. meridionale* collected in May were obtained from brooder houses on May 31. The only blood-sucking insect captured in turkey shelters prior to this date was *E. johannseni*.

Except for the genera *Eusimulium* and *Culicoides,* all insects captured and processed in June were from brooder house collections on June 8. With the exception of 2 pools of 146 *S. rugglesi* collected in May and 1 of 10 *S. venustum* and 1 of 15 *S. decorum* collected in September, all *Simulium* captured and processed throughout the year were *S. meridionale*. All *Eusimulium* collected and processed from July through September were *E. aureum*. The remaining species are listed below according to the months in which they were collected: JUNE—*Culicoides biguttatus, C. crepuscularis, Ae. sticticus, Ae. vexans,* and *An. punctipennis*. JULY—*Culicoides biguttatus, C. crepuscularis, C. haematopotus, C. guttipennis, Ae. cinereus, Ae. vexans, Ae. excrucians, Ae. trivittatus, Ae. stimulans, M. perturbans,* and *Culex pipiens*. AUGUST—*Culicoides crepuscularis, C. haematopotus, C. stellifer, C. arboricola, C. guttipennis, C. venustus, C. variipennis, C. villosipennis, Ae. trivittatus, Ae. vexans, Ae. stimulans, Ae. excrucians, Ae. triseriatus, Ae. sticticus, M. perturbans, Culex pipiens, C. tarsalis, C. erraticus, C. territans, C. salinarius, An. punctipennis, An. quadrimaculatus,* and *An. walkeri*. SEPTEMBER—*Culicoides haematopotus, C. crepuscularis, Ae. vexans, Ae. trivittatus, An. walkeri,* and *An. punctipennis*.

Of the four experimental hosts exposed on May 2, two (a turkey poult and pheasant hen) died on May 4 and 10 respectively. No chicken embryo lethal agents were isolated from either bird. Serum neutralizing antibodies to EEV and WEV were not detected in any birds used as experimental field hosts.

Discussion. Spalatin, *et al.* (1960), found that turkeys of various ages usually responded with the appearance of virus neutralizing antibodies when challenged with 5 to 1,000 LD_{50} units of virus; therefore, serological results obtained by bleeding farm turkey flocks were used to measure flock infection rates under natural field conditions. The number of antibody positive birds showed very little virus activity in 1960 as compared with 1959, but greater emphasis on collecting and processing hematophagous insects during 1960 resulted in the isolation of EEV from 2 pools of *Ae. sticticus,* 1 pool of *E. johannseni,* and 1 pool of *S. meridionale*.

The detected arthropod infection rate paralleled the low incidence of infection serologically determined in turkeys. However, since most simuliids collected and processed for virus isolation attempts were obtained in a related study on their feeding habits and host preferences (Anderson and DeFoliart, 1961), it was estimated that at least half the total number of black flies processed were captured when seeking their initial blood meals following emergence. Similar observations were not made on mosquitoes or ceratopogonids.

Although the significance of isolations from engorged specimens is usually difficult to interpret, 2 of our 3 pools (the 46 *E. johannseni* collected on May 2, and the 15 *Ae. sticticus* captured on August 4) containing engorged flies were captured

after feeding on experimental hosts from which no evidence of infection was obtained. As the third pool of engorged specimens (15 *Ae. sticticus*) was collected in a turkey brooder house, it is not known whether they were infected prior to or after feeding.

The isolation of virus from a pool of 100 non-engorged *S. meridionale* collected in a brooder house on June 8, indicated that this species may serve as a biological vector when exposed to the virus. This fly takes at least two separate blood meals and is one of the known vectors of the protozoan blood parasite, *Leucocytozoon smithi* L. & L. of turkeys (Skidmore, 1932; and Anderson and DeFoliart, 1961). Since field studies revealed two major feeding peaks (May 31 and June 8) of the first generation females, it appears that at least one of the non-engorged specimens collected on June 8 may have served as a biological vector of EEV.

Both blackfly species from which the virus was isolated are ornithophilic. All previous investigations on the potential of simuliids as vectors of viruses in North America have, apparently, involved mammalophilic species (Knowlton, 1934; Knowlton and Rowe, 1934; and Hammon, *et al.*, 1942).

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NOTES ON A MALE *AEDES AEGYPTI* WHOSE TERMINALIUM FAILED TO ROTATE [1]

JACK COLVARD JONES

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In a series of studies on the physiology of the reproductive system of *Aedes aegypti* (Linnaeus) in which many males of the Bangkok strain were being routinely force-copulated with females using a slight modification of the McDaniel-Horsfall technique (McDaniel and Horsfall, 1957), a single, six-day-old male was discovered whose terminalium had failed to undergo the normal 180° rotation (Christophers, 1915). This male was presented successively for five minutes to each of five virgin females of suitable age, but he made no attempt either to

[1] This research was supported by a grant (RG-6021) from the National Institutes of Health, U.S.P.H.S., Bethesda, Maryland. Miscellaneous Article No. 419, Contribution No. 3233 of the Maryland Agricultural Experiment Station, Department of Entomology.

clasp or copulate with any of them. He was then released into a separate cage. Two days later he was still alive with his terminalium still completely unrotated; he was then examined in some detail before being dissected.

The external examination of the unrotated male revealed that he was not a gynandromorph (Figure 1, A). The flagellum portion of his antennae was less densely clothed with long hairs (Figure 1, B) than those of normal males (Figure 1, C). Between the seventh and eighth abdominal segments of the unrotated male

Fig. 1.—A.—Lateral view of a freshly impaled 8-day-old adult *Aedes aegypti* male with unrotated terminalium. The metathoracic legs broke off during handling. B.—View of head of the unrotated male *A. aegypti*. C.—View of head of a normal *A. aegypti* male.

(between these segments rotation of the terminalium takes place), a thin band of pale greyish material (dried secretion?) was visible at the anterior end of the eighth abdominal segment. The basimeres (sometimes referred to as basistyles or side pieces) possessed notably fewer long hairs on the ventral and lateral surfaces than are present on the basimeres of normal males. The ninth segment appeared to be somewhat more retracted than in normal males; the median fleshy lobe of the ninth sternum, for example, could not be seen in the intact specimen.

When the internal reproductive system was extracted from the unrotated male into a drop of *Drosophila* saline (Ephrussi and Beadle, 1936), it was observed to be essentially indistinguishable from that of normal rotated but unmated males of the same age, except that the vasa efferentia were not crossed. The testes were of normal size and shape and possessed a normal number of testicular compartments filled with germinal cells in various stages of development. The terminal chamber of the testes contained actively undulating spermatozoa as did the vasa efferentia. The vasa deferentia and seminal vesicles were full of spermatozoa. The accessory glands were also normal in size, shape, and color. Spermatozoa removed from the seminal vesicles vigorously undulated in the saline in a normal fashion.

This particular abnormality reveals some very interesting facts about the physiology of the reproductive system of the male mosquito. It is evident that rotation of the terminalium is not a prerequisite to the undulations of the spermatozoa in the terminal compartment of the testes or vasa efferentia, nor is it a prerequisite to the descent of the spermatozoa to the seminal vesicles, nor to the secretory activity of the accessory glands. It is also evident that when rotation of the terminalium fails to occur, the male, even though possessing normal looking internal reproductive organs, and only slightly abnormal external organs, makes no attempt to copulate with females and cannot be induced to copulate with them. Unfortunately no observations were made on the question of whether this male was attracted to females. It is conceivable that the decrease in the number of flagellar hairs could have effectively prevented the free male from locating females (see Roth, 1948). Since no information now exists on the factors involved in either the act of clasping or ejaculation, it is not known why this male could not be forced to copulate with females. It is conceivable, however, that the dried secretion between the seventh and eighth abdominal segments and the abnormally retracted ninth segment made copulation mechanically impossible.

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SCIENTIFIC NOTES

Aedes thibaulti in Maryland *

Stanley R. Josephi

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Aedes thibaulti Dyar and Knab has been reported from Cape Henry, Virginia, and from Ellendale Swamp, Delaware (Bickley, 1957). It is reasonable to assume that this species should be present in the section of Maryland which lies between these two locations. However, there is no record of this species having been collected in Maryland prior to this report.

On April 27, 1961, a number of 4th instar larvae and pupae were collected from a hole under the root system of a partially uprooted tree in Dublin Swamp, Somerset County, Maryland. At first, dipping in this hole produced only *Aedes canadensis* larvae but when the samples were taken from the darkest portion of the hole *A. thibaulti* larvae were found. Three adult males were reared from this collection confirming identification of the larvae.

A second collection of this species was made on the same date in a swamp about two miles east of the first site. This time the larvae (2nd and 3rd instar) were found in a large tree hole which was nearly a foot in diameter and extended slightly below the surface of the surrounding terrain.

A third collection of a single adult male was made May 21, 1961, from a resting box in a swamp six miles southwest of Salisbury, Wicomico County, about thirteen miles west of the first site.

During a second visit to the areas in Somerset County, *Culiseta melanura* larvae were found associated with this species at both of the breeding sites. This association has been reported by Shields and Lackey, 1938.

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* Miscellaneous Publication Number 431, Contribution Number 3262 of the Maryland Agricultural Experiment Station, Department of Entomology. [Project H-73-a].

Unusual Larval Mosquito Recoveries From Fire Barrels

R. B. Eads,[1] H. A. Trevino,[2] and E. G. Campos [2]

Among the most important container mosquito breeding habitats around seaports such as Brownsville, Texas, are the barrels of water maintained for fire protection in cotton warehouses. These fire barrels are checked regularly for the presence of vector species, particularly *Aedes aegypti*, and control measures are applied as needed. *Aedes aegypti* may be produced in large numbers in such containers in the absence of control measures. Although the predominant species taken is *Culex pipiens quinquefasciatus*, we are continually impressed by the number of different species found.

Diverse aquatic habitats are likely to harbor characteristic species of mosquitoes; some are limited to a single peculiar breeding environment, while others are found in a great variety of aquatic situations. The ecological distribution of larvae in nature results largely from adult oviposition habits, rather than from inability of the larvae to develop normally in water with a wide range of physical and chemical properties (Bates, 1949). We have previously collected *Culex salinarius, C. coronator, C. tarsalis, C. p. quinquefasciatus, Culiseta inornata, Anopheles quadrimaculatus, A. crucians,* and *A. pseudopunctipennis* from fire barrels in Brownsville, Texas. These adults lay their eggs directly on water surfaces and the presence of their larvae in artificial containers of this type is not surprising. However, our recent recovery of three species of flood-water mosquitoes from fire barrels in a cotton warehouse is deemed of sufficient interest to report. On Oct. 6, 1960, six larvae of *Aedes sollicitans* and two of *Aedes taeniorhynchus* were taken from three fire barrels. A large number of the barrels were also breeding *C. p. quinquefasciatus*. Only a sample of the *Aedes* present was taken for determination. On Oct. 20, four *Aedes sollicitans* and one *Psorophora confinnis* were dipped from a single barrel. Three *Aedes sollicitans* larvae were obtained during a third visit Oct. 24. All were third or fourth instar.

These *Aedes* and *Psorophora* species normally oviposit on moist soil, with the embryos developing to the point of hatching and entering a resting stage until reactivated by flooding. This adaption to temporary ground water would normally pre-

[1, 2] Senior Scientist (R) and Biologists, respectively, Quarantine Station, Public Health Service, U. S. Department of Health, Education and Welfare, Brownsville, Texas.

vent them from breeding in relatively permanent water accumulations.

The fire barrels considered are concrete cylinders with inside measurements of 18 inches in diameter and 34 inches in height. The cylinders are filled approximately once a year with water from the Brownsville water mains. Evaporation in the barrels is at the rate of an inch or two a month. It was theorized initially that adults of *Psorophora confinnis* and the two *Aedes* laid eggs at the water line and that the eggs hatched subsequent to the water levels being raised. However, it was learned that water had not been added to the barrels for at least six months. Consequently, the eggs must have dropped off the sides of the barrels or were laid directly in the water. Travis (1953) reported that eight percent of one lot of *Aedes sollicitans,* submerged in tap water within 24 hours after being laid, hatched within eight days, without an interval of drying.

The 40 fire barrels in the warehouse in question were filled with tap water Nov. 4, 1960, and checked every few days the remainder of the month. No *Aedes* were detected until Nov. 22, when second and third instar *sollicitans* were collected in four barrels. There were only a few larvae in three of the barrels, while the fourth contained more than 100 specimens.

We are unable to advance an explanation as to why these flood-water mosquitoes chose to oviposit in fresh-water fire barrels, particularly in view of numerous roadside ditches in the vicinity which are subject to periodic inundation and which are typical of their normal breeding habitats.

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RELATION OF TEMPERATURE AND HUMIDITY TO WINTER SURVIVAL OF *Culex pipiens* AND *Culex tarsalis* [1]

G. ALLEN MAIL AND ROBERT A. MCHUGH

As an adjunct to studies of the overwintering habits of mosquitoes, observations were made on the survival of females of *Culex pipiens* and *Culex tarsalis* that were collected in a mine tunnel near Farmington, Utah, in January and in December, 1957, and subsequently placed under constant conditions of temperature and humidity. A previous study of this mine (Dow, Mail, and Richards, 1957) reported high mortality in the hibernating mosquitoes. Consequently, both lots of material used in the present study had probably consumed, when collected, more than the normal amount of hibernating fat.

The first series of observations was made on 46 *Culex pipiens* and 47 *Culex tarsalis* that were collected on January 16, 1957. The specimens of each species were divided into 3 aliquots and placed in three constant temperature cabinets held at 27° F., 37° F., and 47° F. The relative humidity in each cabinet was 35–40 percent. The percent mortality was observed at 5, 7, 9, 15, 21, 30, and 40 days. At each observation, the specimens were exposed briefly to higher temperatures to determine which ones were dead. At 27° F., 37° F., and 47° F., all specimens of *C. pipiens* were found dead on the 21st, 9th, and 21st day, respectively; all specimens of *C. tarsalis* were found dead on the 40th, 40th, and 21st day, respectively. The estimated 50 percent survival period (Fig. 1) ranged from 4 to 23 days.

During the following December, further collections of *C. pipiens* and *C. tarsalis* were made in the same mine, and observations were begun in the same cabinets. In this part of the study, the temperatures used were 27° F., 32° F., and 37° F., and the air was specially humidified. The 27-degree cabinet was lined on the bottom and sides with slabs of ice from frozen ponds, and pieces of ice were loosely stacked in spaces not occupied by the mosquito cages. The relative humidity was kept at 84 percent. In the 32-degree cabinet, which had a relative humidity of 87 percent, shallow enameled pans filled with a concentrated solution of zinc sulfate were loosely stacked with one-inch-square wooden strips between them. In the 37-degree cabinet, with relative humidity of 86 percent, there were pans arranged as in the 32-degree cabinet but filled with water. The humidity was measured by a model HA-2 Friez [2] psychrometer which had to

[1] From the Encephalitis Section, Technology Branch, Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Greeley, Colorado.

[2] Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service.

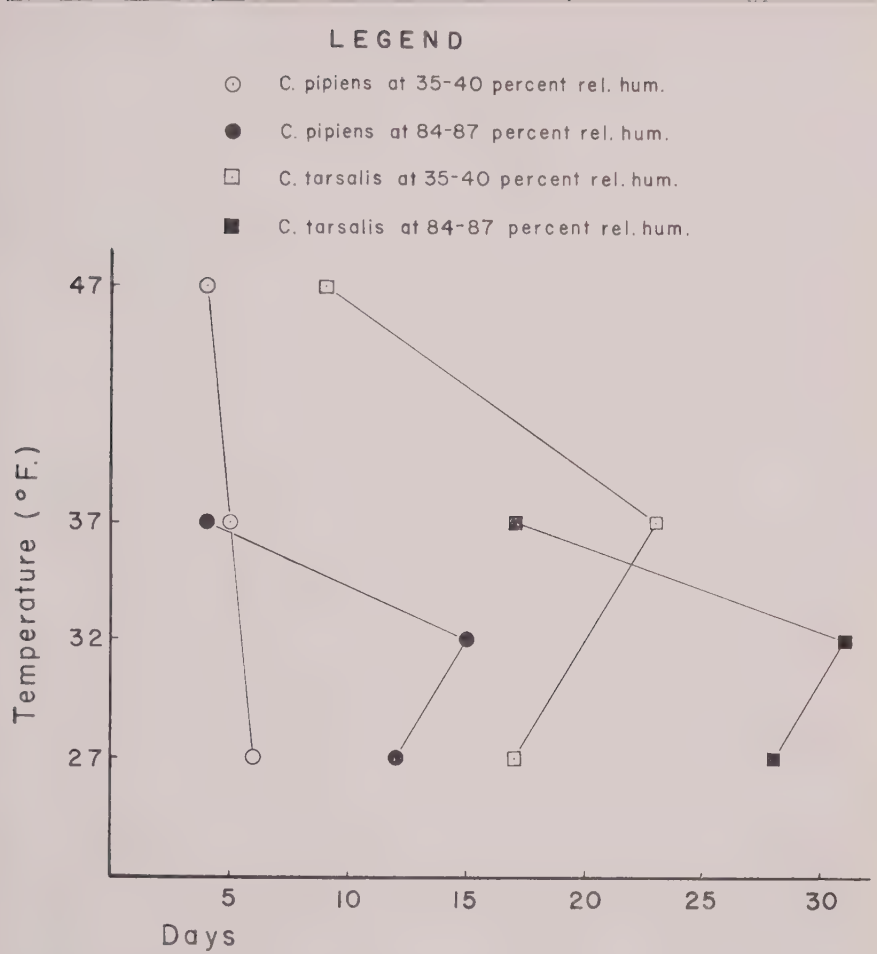

Fig. 1.—Approximate 50 percent survival period of overwintering *C. pipiens* and *C. tarsalis* held at different temperatures and relative humidities.

be left inside the cabinet for several hours before it was used for a reading; otherwise air passing through the apparatus was considerably warmed. All the containers of mosquitoes were examined daily for the first week, and weekly thereafter until all the specimens were dead. As in the previous series of observations, the specimens were exposed briefly to higher temperatures at each examination. The approximate 50 percent survival periods are shown in Figure 1. The maximum longevity which occurred in *C. tarsalis* at 27° F. and 32° F. was over 70 days.

Under the conditions of both of these tests, *C. tarsalis* survived longer than *C. pipiens*, especially at the lower temperatures. At 47° F., there was a relatively small difference (5 days) between the two species in the 50 percent survival period, but at lower temperatures *C. tarsalis* had a 50 percent survival period 11 to 18 days longer than that of *C. pipiens* held in the same range of humidity. This finding, together with the previous observations of Dow *et al.* (*op. cit.*), suggests that *C. tarsalis* is adapted for overwintering at somewhat lower temperatures than is *C. pipiens*.

Comparison of the species at different humidities is complicated by the confounding of month of collection with level of humidity; that is, December specimens might be expected to live

longer than January specimens even if they were not held at a higher humidity. At 37° F., the 50 percent survival period of both species was shorter by at least one day at the higher humidities. At 27° F., however, the 50 percent survival period was 6 days longer at the higher humidity in *C. pipiens* and 11 days longer at the higher humidity in *C. tarsalis*. In general, the observations indicate that overwintering *C. tarsalis* survive longer than *C. pipiens* at any temperature between 27° F. and 47° F., and that the difference is greater at the lower temperatures (27° F. to 37° F.). The tests also indicate that both species reach maximum survival under conditions of high humidity combined with temperatures at or slightly below freezing.

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OPERATIONAL NOTES

The Use of DDVP for the Control of Mosquitoes Breeding in Catch Basins

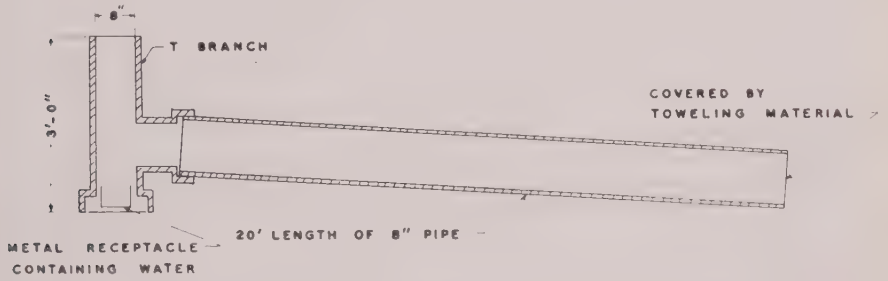
Robert A. Hedeen [1]

In the South Cook County Mosquito Abatement District there are thousands of catch basins (the storm drain inlets at street intersections) that produce great numbers of *Culex* spp. at certain times of the year. To treat these sources the District uses two motorcycles and one truckster equipped for spraying. When inspection reveals breeding in the catch basins they are sprayed with an emulsion containing 5 percent DDT and 1.5 percent lethane (384) at the rate of about one pint per basin. A special nozzle that distributes the spray to all walls of the basin is employed. This procedure has been found to be effective with breeding being controlled for up to several weeks or until a heavy rainfall occurs and washes the insecticides away. On several occasions, however, it has been noted that adults resting some distance back in the outlet pipe of the basin were not killed by the material used.

DDVP (dimethyl 2,2-dichlorovinyl phosphate) is a relatively new insecticide that shows definite promise for use in mosquito control programs. It is a volatile compound, with the vapors having considerable insecticidal properties.

Because of this vapor toxicity it was decided to conduct a series of tests to determine if adults resting well within the outlet pipe of a catch basin would be killed if DDVP was included in our spray mixture. Two simulated catch basins (Figure) were constructed and cages of living *Culex pipiens* adults were introduced into the pipe some 20 feet away from the entrance to the simulated basin.

One pint of 5.0 percent DDT emulsion containing 0.5 percent DDVP was sprayed into the inlet of one of these basins, and one pint of 5.0 percent DDT emulsion containing 1.5 percent lethane (384) was sprayed into the other. Three replicated experiments revealed 100 percent mortality after 24 hours in the basin sprayed with the mixture containing 0.5 percent DDVP. Mortalities of less than 10 percent were obtained in the basin treated with the 5.0 percent DDT-1.5 percent lethane (384) formulation. The Shell Chemical Company furnished the DDVP emulsion concentrate.



Beakers containing numerous larvae of all instars and pupae of *C. pipiens* were placed within a few inches of the caged adults in both test pipes. In both test basins the immature stages were not affected by either of the test formulations.

Emulsions of DDVP and DDT are miscible, and on the basis of these experiments the District plans at this time to instigate the practice of using a formulation of 5.0 percent DDT-0.5 percent DDVP for the control of mosquitoes breeding in catch basins.

Thanks are given to the Shell Chemical Company for making available a supply of DDVP emulsion concentrate.

[1] Manager-Entomologist, The South Cook County Mosquito Abatement District, P. O. Box 30, Harvey, Illinois.

NOTE: Bruce Brockway, now in charge of the Operational Notes section, sent in additional notes which could not be set in time for this issue. They will appear, with others, in December.

ASSOCIATION NEWS

1962 JOINT MEETING
OF THE
AMERICAN MOSQUITO CONTROL ASS'N
AND THE
TEXAS MOSQUITO CONTROL ASS'N
HOTEL BUCCANEER
GALVESTON ISLE, TEXAS
MARCH 5, 6 and 7, 1962

PRELIMINARY OUTLINE OF PROGRAM

Sunday, March 4

9:00 a.m.
Board of Directors Meeting
6:30 p.m.
Dinner for AMCA and TMCA Directors and Wives

Monday, March 5

9:00 a.m.
Opening Session, Preliminary Business Meeting, Invitational Papers
1:30 p.m.
Symposium; Control of Mosquitoes by Non-insecticidal Means
3:45 p.m.
Submitted Papers
6:30 p.m.
Entertainment

Tuesday, March 6

8:30 a.m.
Submitted Papers
1:30 p.m.
Submitted Papers
3:45 p.m.
Final Business Meeting
6:30 p.m.
Hospitality Hour
7:30 p.m.
Buffet Dinner
8:30 a.m.
(Concurrent) Commissioners and Trustees Meeting

Wednesday, March 7

8:30 a.m.
Symposium; Water Management
10:45 a.m.
Submitted Papers
1:30 p.m.
Submitted Papers
3:45 p.m.
Final Board of Directors Meeting

NOTE: Single registration fee will cover all activities—"Texas Style."

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AMERICAN MOSQUITO CONTROL ASSOCIATION, INC.

List of Members, As of August 15, 1961

A

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Adams, Clovis H., 115th & Cottage Grove, Chicago 28, Ill.
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Afridi, Col. M. K., Vice Chancellor University of Peshawar, N.W.F. Province, Peshawar, West Pakistan
Aitken, Thomas H. G., Trinidad Reg. Virus Lab., P. O. Box 164, Port of Spain, Trinidad, B.W.I.
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FOR YOUR CALENDAR

Mosquito Control Association meetings:

California Mosquito Control Ass'n, January 29–31, 1962, San Mateo, California
Virginia Mosquito Control Ass'n, February 23, 1962, Norfolk, Virginia
Texas Mosquito Control Ass'n, joint meeting American Mosquito Control Ass'n, March 5, 6 & 7, 1962, Galveston, Texas
Utah Mosquito Control Ass'n, March 16–17, 1962, Salt Lake City, Utah
New Jersey Mosquito Extermination Ass'n, March 21, 22 and 23, 1962, Atlantic City, N. J.
Illinois Mosquito Control Ass'n, March 30 and 31, 1962, East Hazelcrest, Ill.
Florida Anti-Mosquito Ass'n, April, 1962. Write J. A. Mulrennan, P. O. Box 210, Jacksonville, Florida for exact dates and site.

Also note:

Eastern Branch Entomological Society of America, Annual Meeting, October 30–31, 1961, Lord Baltimore Hotel, Baltimore, Maryland.

Entomological Society of America, Annual Meeting, November 27–30, 1961, Hotel McAllister, Miami, Florida.

NEWS AND NOTES

AUSTIN W. MORRILL, JR.

DR. FRED BISHOPP, WHO CONTINUES HIS ACTIVE INTEREST IN MOSQUITO CONTROL in his new residence in Fort Meyers, Florida, has sent us an interesting clipping from the *News-Press* of that city, concerning a reduction in mosquito control costs. WAYNE MILLER who runs the mosquito abatement program there recommended that for fiscal year 1962 the budget could be *reduced* 12.7 percent by following certain long-range plans which would make for greater efficiency and Dr. Fred pointed out that there is no surer way of making the top headlines in your local paper than to recommend a reduction in taxes appropriated for yourself. But doing this while at the same time purchasing needed (and more efficient) heavy equipment and basic apparatus, takes some little doing. Dr. Fred says Wayne has a fine and well balanced program and that in itself is a wonderful bit of good public relations for mosquito control everywhere. Considering how many of our own good constituents visit Florida and Fort Meyers every year, perhaps we all owe Wayne a big vote of thanks. (The same paper had a picture of Wayne's young son, T. Wayne Miller III, who was the Junior King of the colorful Thomas A. Edison Pageant of Light and showed an aplomb at it, at the age of 6, which many an older hand might envy.)

The dedication of the water control structure on Sanibel Island (Lee Co., Florida) was also quite an affair, according to Dr. Fred, with an elaborate and interesting, as well as instructive, program. DR. MAURICE PROVOST was programmed as principal speaker and attendance included mosquito control people from all over, many from other states and areas.

PERHAPS MOST AMCA'ERS are by now on the mailing list of the *Vector Control Newsletter* which the USPHS issues from time to time. For those who are not, however, we'd like to repeat a few little items we found interesting. F'rinstance, CLYDE F. FEHN recently figured up his costs on Chatham County (Georgia) Mosquito District's use of paris green pellets, at the end of the third year of use around Savannah. Excellent control was obtained of *Aedes, Psorophora* and *Culex* larvae. The pounds of material used rose from about 7,000 in 1958 to 86,575 in 1960. Half were applied by hand at a cost of $3.00 an acre, while the other half, applied by airplane, cost about $1.50 an acre. Clyde's report gives a lot of other fascinating details we won't go into, such as other equipment used, dispersion patterns and such.

THE FIRST MOSQUITO CONTROL DISTRICT IN COLORADO AND THE FIRST ORGANIZED MUNICIPAL VECTOR CONTROL PROGRAM IN SOUTH DAKOTA are also reported in the *Newsletter*. RALPH C. BARNES reported the first one, which will begin operations this year (perhaps already has?) in the Animas District, including the city of Durango and areas adjacent to the Animas River. The enabling legislation was passed in 1959. The second of the two "firsts" as reported by Mr. C. C. Carl, was organized in the City of Sioux Falls, So. Dakota and a trained entomologist was hired in 1960 with the expectation of starting control operations this year, following intensive population studies made last year. In this program, the original intention of sticking to adult control was successfully redirected into the sounder channels of larval control, and other municipalities who have previously had less organized, more one-sided programs are watching Sioux Falls' trailblazing in the Dakotas.

DR. DICK HAYES REPORTS IN THE SAME ISSUE ON THE ISOLATION OF WESTERN ENCEPHALITIS VIRUS from mosquitoes in Massachusetts. This was done from *Culiseta melanura* and is a first we aren't glad about, though glad to know about it, if it has to be. Four pools of mosquitoes, one deplete and three engorged were found positive, the three latter ones being from grackle-baited traps.

VMCA'S *Skeeter* is also a mighty interesting source of stimulating news, as you all know, and in addition to what we told you last issue about VMCA's Annual Meeting, *Skeeter* informs us that they discussed the Association's highly successful mosquito light trap program. Twenty-three traps are operated by the Association for six Commissions and during the period May to September of last year 64,607 identifications were made and reported on. ROLLIE DORER, who has long been so active in promoting good mosquito control, assisted the town of Jarratt in establishing a joint control program with the Johns-Manville Company, following a mosquito survey. A similar survey has resulted in plans for a program in the city of Alexandria, one of the important suburbs of Washington, D. C. (Virginians like to think of Washington as still the lusty young neighbor of long-established Alexandria, which was once the "town" for Mount Vernon.) If it seems odd that a state having such fine and long-experienced mosquito control districts as Virginia has, should also be engaged in *establishing* one in one of its major areas, remember that Virginia is also wedded to the concept of Local Option and Alexandria, though embattled, has been able to hang onto the Dignified Pace.

Rollie Dorer Was Also Responsible for Providing the Impetus to the Establishment in Virginia of a Study Grant From USPHS on Rocky Mountain Spotted Fever. This isn't mosquito control but we thought you'd be interested. Charles Gerhardt was instrumental in the project as well and work, which started in April, will carry on for a projected period of four years.

Our Recent Remark Congratulating Amca-Esa'ers on Amca Having Made a Clean Sweep of the Offices of Section D of Esa Drew an Anguished Howl From Bill Rogoff, Section D's Secretary. "Don't," he cried, repeat "Please Don't withdraw your comment of last summer that all AMCA'ers 'should be in Section D of ESA.' While a goodly number are already members of this Section, there is room for many more." Dr. Rogoff says he has just compiled a membership list of Section D and was astonished at the number of members of AMCA who are not affiliated with the *mosquito control section*. He points out that affiliation is simple and can be had by dropping a card to Bob Nelson, at ESA Headquarters, requesting the "desired (and desirable) status." Come*on* there fellows . . . time's a-wastin' and the cost of penny post cards is about to go up to four cents. You cain't ever do it so cheap again!

We Don't Want to Seem to Overlook New Members From Such Old Strongholds as Pinellas County (Florida) Where New Member Louis L. Fernald hails from but we guess we can't call out *welcome aboard!* to all the new members individually, on account we have trouble keeping up with so many. But when they come from fur places like our 49th State, we think we ought to give a big *welcome aboard!* to Francisco C. Go, Satyendra P. Mathur, and Warren Griffin, all of Juneau. It's good to see mosquito control going ahead strongly in this wonderful vacationland so long famed for hunting, fishing and mosquitoes, now that the country is settling down to prosaic statehood and other workday affairs.

From Ted Raley Also Comes the Information that some changes of address have taken place since June. Robert W. Lake and Richard F. Darsie are now at the Department of Entomology, University of Delaware, Newark, Delaware. John O. Stivers has changed from USOM/Nicaragua to USOM/Ecuador, % American Consulate General, Guayaquil. Robert T. Taylor has left Bogotá for Pan American Sanitary Bureau, P. O. Box 1193, Paramaribo, Surinam, and James D. Williams has gone to the USPHS Sanitary Engineering Center, Cincinnati, Ohio. James L. Hawkins has gone to Coco Solo, Canal Zone (P. O. Box 274), Capt. John E. Scanlon has entered on a new assignment with the U. S. Component, SEATO, % OUSARMA, APO 146, San Francisco, California (that ain't near *me*—Ed.), Keith B. Neely has left California (!) for 2036 Leroy Apts., Apt. 4, Klamath Falls, Oregon, and Dr. Julian De Zulueta has gone from Uganda to the Malaria Eradication Division, World Health Organization, Palais des Nations, Geneva, Switzerland, and we bet *that* climate is a big improvement. How about a few words from all you guys as to your new assignments and what you're doing that isn't classified and how you like it (if that's not classified either!).

Constant Readers Are Aware that We Have Sent to Us Monthly Reports of Many of the Mosquito Abatement Districts Around These United States and we hope you all know that we'd like to have more of them from more of you. Not every remark you make to your trustees and stockholders is of universal interest, naturally, and some of it you may think is too elementary a sort of background indoctrination to go over with our *sophisticated* audience . . . but you'd be surprised and we'd be delighted to have demonstrated the wide interest there is in each other's doings. Trouble is, most folks want to get letters but don't want to write them. Well, some of our Constant Correspondents have made some cogent remarks this time. Don Murray, for instance, in his Delta (California) Mosquito Abatement District's Operational Report, noted that *Aedes vexans,* a prime cause for the establishment of his District in 1922, but a pest of minor importance of late, is recently appearing in quite untypical locations . . . irrigation tailings inside walnut groves. Can this augur a move by *vexans* toward becoming an urban species in retaliation for the constant reduction of its original breeding zones in natural river-bank flood waters?

Don Rees and Glen Collett Reported in Their Recently Issued Summary of Salt Lake City's Thirty-First (1960) Season that along with improved water management, a variation of the pre-melt application of insecticides on snow was successful when they applied insecticides to dry ground of duck ponds prior to their being flooded. Approximately 500 acres were pretreated with DDT at the rate of 1 pound per acre and results were very satisfactory, with few mosquitoes appearing until four to six weeks after the flooding.

Bob Hedeen's South Cook County Mosquito Abatement District's Annual Report is not only handsome but has an excellent feature: a discussion of What Do We Do in the Winter? Listing eight activities which are carried on during the cold weather, including soil sampling, mapping, maintenance of equipment, pre-hatch treatment and research, the report gives a rather thorough description of what each entails and the reason such an activity is important to a properly grounded program. A good educational example . . . some of us sort of tend to take it for granted without explaining ourselves fully to the interested bystanders, *we* think.

THE UNIVERSITY OF MARYLAND now receives and pays for FIVE subsciptions to *Mosquito News*. We don't know who's to "blame" for this fine record, but we think it *is* some sort of a record and want to say Congratulations U. of M. (and thanks, Bick!).

AND ON THE HIGHEST AUTHORITY IT IS A WELL-FOUNDED RUMOR THAT CMCA (California, that is) will hold its 1962 meetings at the Villa Hotel, San Mateo, on 29 to 31 January, 1962. AMCA'ers welcome. Remember you have a date in California!

BILL CANTELO IS DESERTING THE BOONDOCKS OF GUAM FOR THE LUSH GREEN FIELDS and emerald jungles of Thailand. After a six week R and R in Boston (this, in August, is Rest and Recuperation from *Guam?*), Bill goes to Washington (hotter and hotter) for indoctrination and thereafter will be at ICA, Bangkok. He'll be working mainly with agricultural-type bugs, but will still keep a weather eye out for our trusty friends.

CHET ROBINSON has been appointed Chairman of the Inter-Agency Fly Committee of Alameda (California) County and of the Alameda County Citizens Fly Control Committee. The latter is being enlarged by reason of a fly survey being conducted by the State Department of Public Health. Alameda County, like many others throughout the country, has discovered that fly control and urban mosquito control often go hand in hand, and vigorous efforts are being made to attack the problem before it reaches the magnitude of another city's, which recently made national headlines.

WELCOME ABOARD TO DR. IN HO CHU, Professor of Preventive Medicine at Soo-Do Medical College, Seoul, Korea. If we are remembering correctly, this site is one from which mosquito control was carried out when we were last in Seoul, during the Late Unpleasantness, and so it seems like a fitting address to which to be sending *Mosquito News*.

WE'VE QUOTED GEORGE THOMPSON A GOOD BIT HERE WITHOUT EVER GIVING YOU THE WHO'S WHO LOW DOWN on him. For those of you who don't know him, George is the energetic manager of the Jefferson County (Texas) Mosquito Control District. He was born in Providence, Rhode Island, in 1909, and went to school in that city. In 1932 he received his BS from the University of Rhode Island and in 1934 his MS, after which he was Assistant State Entomologist for some four years. The U. S. Department of Agriculture claimed him for the next four, moving him around in Texas, Louisiana, Alabama and Mississippi but not nearly so much as the USPHS was to do after *it* got him. TOM (TRAVIS E.) MCNEEL hauled him into the MCWA (at his urgent request, since the Navy objected to his wearing glasses) as a 1st Lieutenant, and shortly thereafter he was busy with mosquitoes in Jamaica, BWI. Since the war, PHS work took him to San Juan, Puerto Rico, Corpus Christi, Texas, Kearney, Nebraska, Greeley, Colorado, Oklahoma City and Austin, Texas, from which he was called to head up the newly formed District at Nederland. While in Jamaica he received two signal honors, the Army Commendation Medal and *Mosquito News*, Vol. 4, so George is a real Early Member. Besides a considerable number of publications in *Mosquito News*, George has authored papers on isolation of WEE virus from *Aedes dorsalis* in the Midwest, malaria transmission in Jamaica, the effect of DDT on scorpions and other unusual subjects. He is married and the father of a daughter who is a Junior at the University of Texas.

CAPT. CYRIL J. HODAPP, MSC, USAF, was born in 1927 in St. Louis, Mo., but lived most of his life in Van Nuys, California, where he went to school. In 1945 to 1947 he was in the Navy and after receiving his discharge attended Loyola University in Los Angeles, where he received his BS in 1951. His MS in entomology was from the University of Maryland in 1960. In the meantime he had joined the Air Force and been assigned as entomologist of the 4th Epidemiological Flight USAFE, consultant of the Strategic Air Command, and entomologist of the 3790th Epidemiological Laboratory, Lackland AFB, Texas, where he now is. His European tours took him for 5 months into French Morocco, for 3 months into Libya and a number of other exotic places besides the usual but fascinating corners of the Old World. He is a member of a number of societies, including the ESA, the Washington Ent Soc, and the American Society of Tropical Medicine and Hygiene. He is the father of six children, thus eclipsing KEN KNIGHT, and his hobbies are diving and swimming, painting, woodwork, and other shop crafts; probably some slight connection to the family he heads. He doesn't mention PTA or Cub Scouting, but we bet they're there someplace.

LARRY LEWALLEN was born in 1921 in Tulsa, Oklahoma but also went to school in California, receiving his AB from San Jose State College in 1948, with Honors in Biology. He then received his MS from the University of California, in 1949. He also was in the Air Force, when it was the US *Army* AF, in 1942 and 1943. He spent from 1949 to 1956 at the University of California at Riverside and the next two years at the Los Angeles campus, teaching, and then heard the call to join the research program of the Bureau of Vector Control of the Calif. State Dept. of Public Health. Since then his work on mosquitoes has become known to all. He is a member of ESA and Sigma Xi, as well as of AMCA, and his hobbies are photography and fishing which, like TOMMY MULHERN, he finds himself ideally situated for, Fresno being close to both

the Sierra and the Coast Range (and the Coast). Well, pretty close; you know how they look at these things in California.

DALE JENKINS' Who's Who biography has to be sort of short because he works in a place which they won't even let you mention without Q clearance, almost. However, we can tell you, as Drew Pearson says, that Dale was born in Wapakoneta, Ohio, in 1918, was married in 1942 and has four children. He took his B.A. and M.A. in 1938 and 1939 and later his Ph.D., in 1947, at Ohio State University. He was a Captain in the Army from 1945 to 1947 the last year of this being at the Army Chemical Center, Maryland, where the Army Environmental Health Laboratory is located. In the summer of 1947 he did mosquito research in Alaska, in 1948 did consultant work for FAO, of the United Nations, and in 1949 and again in 1956 did mosquito research with the Canadian Defence Research Board, up around Ft. Churchill, Manitoba, on Hudson's Bay. He remained at AEHL as entomologist until 1952, when he was transferred to Ft. Detrick, Maryland, as Chief of the Entomology Branch and Deputy Chief of the AS Division.

HOLD THE PRESS ITEMS. JOHN BRAWLEY's new address is 1540 Manchester Rd., Chico, California. NORMAN GRATZ has left Jerusalem to be Project Leader, Insecticide Testing Unit, WHO, % Federal Malaria Service, Yaba, Lagos, Nigeria. JOHN R. ANDERSON, recently of Madison, Wisconsin, is now at the Dept. of Entomology and Parasitology of the University of California, Berkeley 4. CAPT. LEONARD TRAGER has left the 5th Epidemiological Flight to go to the University of Maryland at College Park. And new members from abroad include CONRAD BEHRE of Hamburg, Germany, C. J. GARLAND of New Brunswick, Canada, and RAYMOND SHEPPARD of the Engineer Section, 7th Logistical Command, APO 47.

DOYLE REED, papa of mosquito control in the Engineer services of the U. S. Army—and Uncle to much of the military entomology program—was recently given recognition for the role he has played in making the Armed Forces insect control conscious. He was awarded the Sustained Superior Performance Award, chief ribbon granted to civilians by the military, together with a nice purse. Lt. Col. Reed, MSC-USAR (Ret.), has a chestful of ribbons and of course money isn't *everything*, but still it does lend a certain nice emphasis to a well-merited Badge.

ONE LAST ITEM. HAROLD GRAY recently was elected an Honorary Member of the Western Branch of the American Public Health Association on the occasion of his retirement as Editor of *Western Public Health*, in which capacity he had served for some nine years, despite that period of ill health a few years ago which had us all scared.

TOO LATE FOR INCLUSION

Reference to the minutes of the Annual Meeting which appear on page 144 of the June issue will show that there was a discussion of the suggestion that the name of this journal *Mosquito News*, be changed to something "more suitable." This subject is certain to come up again at the next Annual Meeting, probably with the request that some definite action be taken. The editor has received two well written editorials on the subject by prominent members of the AMCA, but with opposite viewpoints. These editorials were received too late for inclusion in the current number. It is planned to print them in December. In the meantime the editor would be glad to receive other comments on this question.

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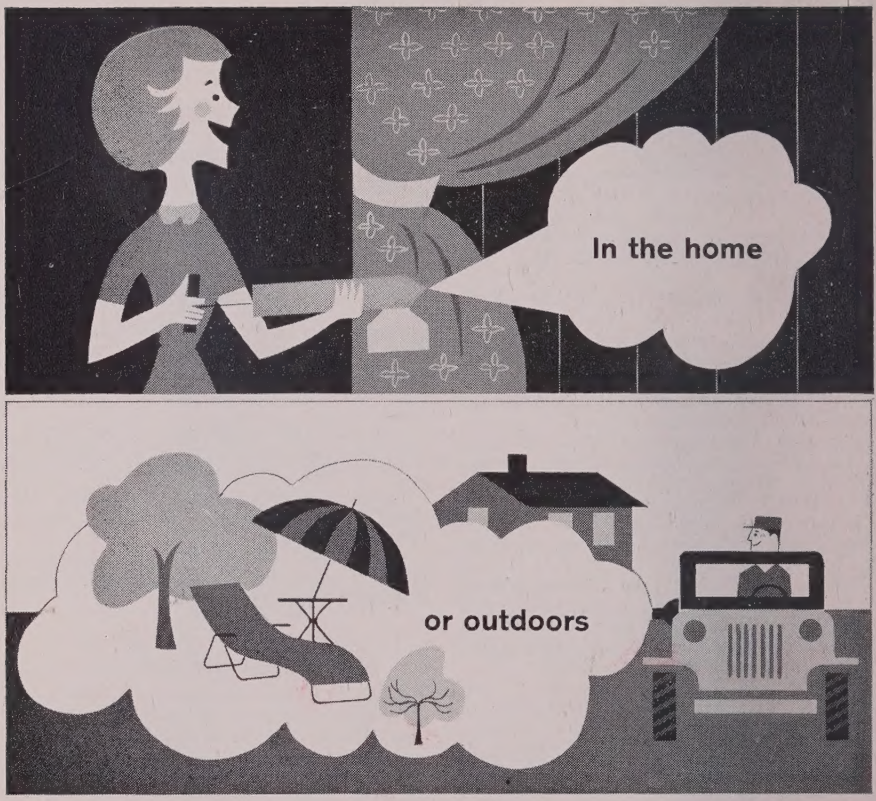
ERRATA

In Mosquito News 21(1):71 under Behavior, Biology and Ecology section, change volume number of reference by O'Gower to read 85; ibid. 21 (1):72–73. Under Taxonomy section change volume number of Linn. Soc. N. S. Wales to read 85 in references by Dobrotworsky, Klein and Marks, and Marks.



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